## EXPERIMENTS WITH ALCOHOL AND WHITE RATS

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SINCE the familiar paper by Elderton and Pearson ('10) upon the physique and ability of children from alcoholic parents, much discussion has taken place on the relation of parental alcoholism to the condition of the offspring. A small proportion of this has been based upon experimental work with animals, as that of Stockard ('12 and '13), Stockard and Papanicolaou ('16 and '18), Nice ('12 and '13), Pearl ('17), and Arlitt ('19). From such studies there should be no hope of obtaining an immediate analysis of the human problem. In so far as alcoholism in man is sociological, involving factors of family life, environment and education, no study of laboratory animals can have significance. The way such studies may have a bearing upon the human problem is through the revelation of general biological reactions that may in all the animals available for study, be found so invariable that it becomes safe to conclude that they appear in man as well. How far the specific findings herein reported for white rats may apply to different animals is a matter for experiment and not conjecture. But even were such a biological analysis secured, the other phases of the human problem would not be solved.

From the data at hand are there any indications of general biological reactions that may have significance for all animals? Stockard and Papanicolaou, with guinea pigs, found that alcoholization of parents gave un-

favorable results in the offspring; Pearl reported generally favorable results in the offspring of treated fowl; Arlitt reported unfavorable results from mild doses on rats, while Nice, also with mild doses, found his test mice slightly better in growth and fertility but less active, as measured by the revolutions of the revolving cages in which they were placed, than the controls. Earlier, Hodges ('03) had found the viability of puppies reduced by the treatment of their parents; the treated dogs were less active and more susceptible to distemper; Laitinen ('08) reported high rates of death at or soon after birth of guinea pigs and rabbits from treated parents.

Accepting these general statements as correct, there appears to be no obvious uniformity in the results obtained by different investigators. But this lack of uniformity may be only apparent; it is possible that not all the results as presented will be confirmed by subsequent investigations since none of the experiments reported have escaped unfavorable criticism from some standpoint. Alcoholism has such a multiplicity of aspects that it is a matter of great difficulty to arrange experiments concerning its effect on the offspring of treated animals that will be beyond criticism. For technique satisfactory to a physiologist may involve serious errors in the eyes of a psychologist, while the experiments of both may, to a geneticist, seem to have weak points. Until alcohol studies meet the requirements of all critics no final conclusions can be reached. In problems involving comparisons between experimental and control individuals the nature of the controls is no less important than the comparison itself. However true this appears to be for all experimental work, it is surprising to note that the main adverse criticisms of the experimental studies of the influence of alcohol upon the offspring have been aimed at the controls.

In spite of the general lack of uniformity in the results as they stand, at least one criterion appears to show consistency. This is the reproductive capacity of the treated

individuals.  All the experiments appear to indicate an immediate reduction in the number of offspring.  The uniformity of this result tends certainly to increase its value as a *general* result; but even so, as long as the controls are subject to criticism, the apparent consistency may be due to the controls and not to the regularity of the reactions to alcohol.  For a single result can not at the same time prove the reliability of the controls and the results of alcohol treatment.  It is hoped that the controls employed in the following experiments will be found to approach the ideal of satisfying all requirements.

## METHODS

In 1914 an investigation was undertaken upon the influence of alcohol on the untreated descendants of white rats with the primary object of studying the behavior, or learning capacity, in different generations.  In the summer of 1917 war conditions necessitated repeated reductions of the stocks until, by the end of the next year, the material was completely lost.  This calamitous termination of the work must be borne in mind, for, in spite of the final nature of this report, the data come from an investigation that was not completed.

*Material and Breeding.*—The rats employed belonged to four strains; three of these strains originated respectively from three pairs of rats in the Wistar Standard Stock, the fourth strain had been bred in this laboratory for three generations.  All matings were between full brothers and sisters.  When 28 days old the litters used to start these experiments were divided into two lots on the basis of equal weight and equal numbers of each sex; one of these lots was used as controls, the other was treated.  All matings were between the original treated males and females or their descendants, or between the original control males and females or their descendants. In each generation the control matings parallel those of the descendants of the treated animals, so that each group of test animals in each generation had its own particular

group of controls. Since inbreeding was the rule, the closest possible relationship for the tests and controls in the successive generations was secured; they came from a single pair of grandparents or great-grandparents, and were thus raised at the same time, and after the same number of generations of inbreeding.

*Treatment.*—The treatment of these rats was by means of the inhalation method, now made familiar by the work of Stockard and Pearl. The rats were placed in closed tanks filled with alcohol vapor; these tanks have been described in detail elsewhere (MacDowell and Vicari, '21). Beginning at weaning (28 days) the rats to be treated were placed in the tanks for 30 minutes a day for 7 days. After this the duration of the daily treatment was measured by the reactions of the animals; for the next 14 days the rats were left daily in the fumes until they were obviously under their influence; subsequently the rats were left each day until they were completely anesthetized. This required from three to four hours for the older rats.

*Criteria.*—The term *treated* is used to indicate rats that were placed in the alcohol fumes after birth. The following generations are herein reported: (1) the treated rats, (2) the treated offspring, (3) the untreated offspring, (4) the untreated offspring of (3) (second untreated generation following one treated generation). For these rats the following types of data are given: the behavior in the maze, as measured by time per trial; behavior in a multiple choice apparatus, measured by the number of correct first choices; fertility, judged by the size of the litters and the number of litters; body weight, as judged by growth curves based on weekly weighings.

### Maze–Behavior

*Apparatus and Training.*—The maze used in this study was built according to the details given by Watson ('14); namely, a concentric arrangement of five alleys with doorways and blind alleys so arranged that the true path from

the outside to the center required a rat to turn alternately
to the left and the right at successive doorways. A rat's
training was started at the age of 56 days, after prelimi-
nary feeding in the center of the maze on each of the 7
preceding days. Three successive trials a day were given.
After the first and second trials the rat was removed
from the center as soon as it had tasted the food (bread
and milk) which was always found there; after the third
trial, it was allowed to eat for five minutes. This train-
ing was given for eight successive days. The observa-
tions were so automatic that there was practically no
possibility that the results were being influenced by an
unconscious bias on the part of the observer. In the
case of the treated rats the alcohol was given each day
following the trials in the maze.

*Results.*—The average time per trial for each day of
the training of the different groups of rats is represented
in Fig. 1. The test rats, whether actually treated, or
the descendants of treated rats, are represented by the
broken lines, and their respective controls by the solid
lines. The numbers of rats included in the different
curves, beginning at the left, are as follows: 55 treated
rats and 62 controls; 46 tests and 48 controls; 25 tests
and 25 controls; 8 tests and 20 controls. The broken
lines tend to lie above the solid lines. The tests tend to
give higher time averages than the controls, that is, the
tests took longer time to run a trial. The inferiority
shown by the treated offspring from treated parents
(fourth pair of curves), and by the untreated offspring
from untreated parents and treated grandparents (third
pair of curves) is of the same order of magnitude as
that shown by the treated animals themselves; untreated
offspring from treated parents show less inferiority than
their own untreated offspring. Considering the signifi-
cance of the differences between the tests and controls
for each day independently, the following results are
found: the differences between the tests and controls are
over three times their probable errors on five days in the

first pair of curves, on no day in the second pair, on four days in the third pair and one day in the fourth pair. All the significant differences favor the controls. However, more important than the significance of individual
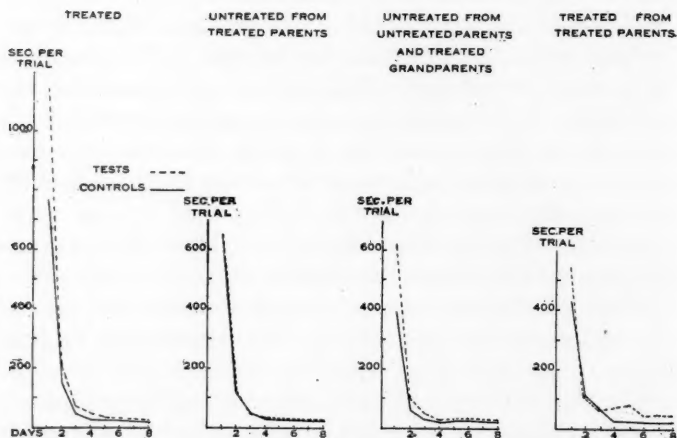
## MAZE

## TIME PER TRIAL



Fig. 1. Comparisons of time averages in four groups of rats—those treated, their treated and untreated children, and their untreated grandchildren. (Data for the third set of curves taken from MacDowell and Vicari, '21, p. 233.) Broken lines tests, solid lines controls.

differences as measured by the probable errors, is the agreement in the direction of the differences on successive days. The fact that the differences on eight successive days lie in the same direction probably has more significance than that half of these taken separately may be significant as judged by their probable errors. Considering the signs alone, in all the curves there are three out of the 32 points of comparison showing the test averages lower than the controls. One of these cases is on the third day of training of the untreated rats from treated parents, the other two cases are on the second and third days of training of the treated rats from treated parents. If chance alone is working, the probability of

eight days giving differences in the same direction is the same as the probability of eight coins coming down all heads; in the long run this will happen once in 256 tosses. The chances of seven out of eight, 1 to 32, of six heads out of eight, 1 to 9. Carrying this comparison further by considering all the generations together, the chances of finding three cases favoring the tests out of thirty-two are in the neighborhood of 1 to 860,000. From all this it appears that the test rats are different, as a group, from the controls. Apparently the only difference between the tests and controls that could explain this result is the alcohol treatment given directly, or in the ancestry of the test rats; this leads to the conclusion that the difference in behavior is due to the alcohol treatment.

### Behavior in the Multiple Choice Apparatus

The difference in the behavior of the tests and controls in the generation of the untreated offspring of treated parents is further shown by the training on the multiple choice apparatus. This is the only generation from which sufficient data were gathered for the analysis of behavior on this apparatus.

*Apparatus and Training.*—The apparatus used in this training consisted of a linear series of nine compartments, with front and back doors operated at a distance by the observer (see Yerkes, '21, for history and uses of this apparatus). Different sets of front doors were opened for the successive trials and the rat was given its reward of food by raising the back door when it entered the " correct " compartment. The " correct " compartment was the one at the extreme right or left (according to the problem) of the series with open front doors. In successive trials, therefore, the correct compartment was never the same one, and the solution of the problem did not depend upon the repetition of a regular kinesthetic habit. The steps in the training were these: at the age of 65 days the preliminary training

was started; on the first two days the doors were all left open and food was exposed to view in every compartment; the rats in groups of five or so were left to run at random in the apparatus. On the second two days the front doors were all open as before, but the food was concealed by covers fastened to the back doors, and when a rat entered any compartment the food was revealed by opening the back door; the rats were run singly on these two days and given ten such feedings a day. On the last two days of the preliminary training only the regular series of doors were opened, but the rats were fed on entering any compartment (20 trials).

*Right-hand Problem.*—In the first problem the rat was fed only when it entered the right-hand compartment of any set-up (those open in any trial); after wrong choices the rat was confined in the compartment for half a minute, and then, by raising the front door, was permitted to make further choices (10 days, 100 trials); next, the same problem was given with a different series of open doors (2 days, 20 trials). Further training was given in the form of a problem in which the correct door was the open one at the left end of the open series, but the results from this problem are so complicated that they will not be treated at this time. The main reason for this complication is the fact that at the end of the time allotted for the mastery of the first problem the test and control rats exhibited different degrees of perfection; some had made considerable progress in learning, while others had made very little advance. Accordingly, when the reverse problem was given, those that had learned the most were handicapped by the habit already acquired, while those that had not formed the required habit in the first problem were able to progress more rapidly in learning the second problem.

*Results.*—From a study of the individual reaction tendencies as revealed in the last two days of the preliminary training before the problem was presented, and in the regular training after the presentation of the prob-

lem necessitated the use of the trial and error method
of finding the correct compartment, it appeared that the
test rats continued the same tendencies in the regular
training that were initiated in the preliminary training,
but the controls, on the other hand, modified their re-
action tendencies as soon as the regular training was
started. This result is brought out by the curves in Fig.
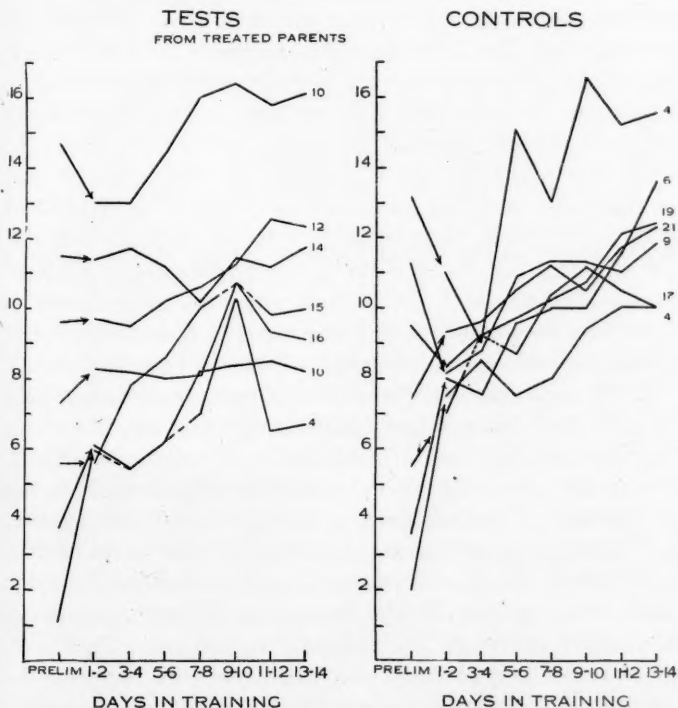


Fig. 2. Showing the relationship for rats from treated parents between pre-
liminary and subsequent performance in the multiple choice apparatus. Aver-
age numbers of correct first choices are shown for each successive set of 20
trials. The rats have been classified into groups according to their preliminary
records. The figure shows that the behavior of the tests in the preliminary
trials is a fairly good index of their behavior in the regular training, but the
behavior of the controls in the preliminary trials gives very little indication
of the later behavior.

2. The test rats have been classified into seven groups according to the number of right-end choices in the last twenty trials of their preliminary training. The first points of the lines given for the tests indicate the average number of right-end choices made by the rats in each of the groups in the preliminary training; the following points give the average numbers of correct (right-end) choices made by these same rats in successive sets of 20 trials in the regular training. Since the procedure in the regular training is essentially different from that in the preliminary trials, the lines connecting the first and second points are drawn as arrows. The numbers at the ends of the lines give the numbers of individuals included in each group. The arrangement of the controls follows the same plan. Whereas the curves for the tests show a general parallelism, those for the controls are, with the exception of the group of four rats whose preliminary training gave between 12 and 14 right-end choices, relatively independent of the preliminary records. This matter can be brought out more clearly by a study of the coefficients of correlation between the preliminary record of each rat and the trials in the regular training. When the correlation coefficients between the preliminary records and the first 20 trials in regular training, and between the preliminary and the second twenty trials in regular training, etc., are calculated, the figures in Table I are obtained. In every case the differences between the coefficients of the tests and controls (fourth column in Table I) show that the tests have higher correlations, and in all but the correlation between the preliminary trials and the last set of twenty trials in regular training, the differences are statistically significant. These results indicate that there is a real difference between the tests and controls in the way they react to the necessity of using trial and error methods; this may be due to a difference in responsiveness to changes in the situation. The tests appear to be less responsive to the changed procedure, since they continue the same general behavior

as in the preliminary training, whereas the controls modify their behavior as soon as the change is made in the procedure.

TABLE I

CORRELATION COEFFICIENTS, SHOWING THE DEGREES OF SIMILARITY BETWEEN
THE NUMBER OF RIGHT-END CHOICES IN THE LAST 20 TRIALS OF
THE PRELIMINARY TRAINING AND THE CORRECT CHOICES IN
EACH SUCCESSIVE SET OF 20 TRIALS IN THE SUBSE-
QUENT TRAINING IN THE MULTIPLE CHOICE
APPARATUS.

| Trials Correlated | Correlation Coefficients | | Difference | D/P.E. |
|---|---|---|---|---|
| | Tests | Controls | | |
| Preliminary by 1st 20 trials.... | .688±.039 | .139±.063 | +.549±.074 | 7.4 |
| by 2d " " .... | .628±.045 | .072±.075 | +.556±.087 | 6.3 |
| by 3d " " .... | .592±.048 | .339±.067 | +.253±.082 | 3.1 |
| by 4th " " .... | .444±.060 | .070±.075 | +.374±.096 | 3.8 |
| by 5th " " .... | .432±.061 | .101±.074 | +.331±.095 | 3.4 |
| by 6th " " .... | .489±.057 | .049±.075 | +.440±.094 | 4.6 |
| by 7th " " .... | .342±.061 | .212±.072 | +.220±.094 | 2.3 |

All the coefficients are positive; the plus sign is used before the differences to indicate that the coefficients for the tests are higher than the corresponding ones for the controls.

In view of the above, the direct comparison of the averages of the tests and controls in regular training would lead to error unless the average performance in the preliminary training happened to be the same for both sets. In the long run this would undoubtedly be the case, but, as it happens, the averages for the tests and controls do not agree in the preliminary training. However, it was found that this difference depended upon the rats with strong right- or left-hand tendencies, for if these (those tests and controls with more than 12 or less than 3 right-end choices in the preliminary training) be omitted, the average of all the rest of the rats was the same for the tests and controls. Using the rats whose preliminary records showed between 3 and 12 inclusive right-end choices, the averages for the curves in Fig. 3 were obtained. Starting with the same average tendency to enter the right-end compartment in the preliminary training, the controls

increase the number of correct first choices more **rapidly**
than do the tests, and as the difference between the **av-**
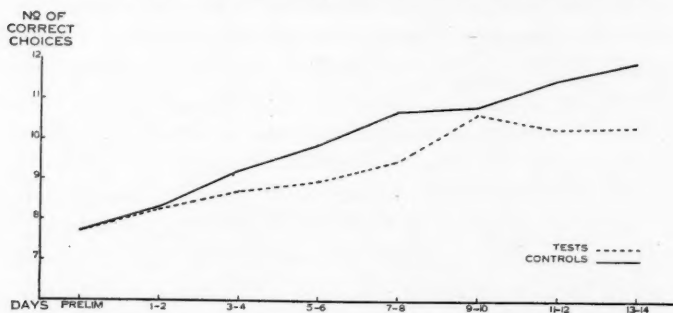erages increases it becomes statistically significant.



FIG. 3. Average numbers of correct first choices in the multiple choice apparatus, in the preliminary training and in the following pairs of days, when only those rats are included which made from 3–12 correct first choices in their preliminary training, *i.e., eliminating* rats with strong tendencies either to choose or avoid the correct door, before regular training began. In this way the preliminary averages of the tests and controls are brought together and it becomes possible to compare the averages in the regular training.

Granting that the controls are adequate, the data on
behavior indicate that a modification has been **brought**
about by the alcohol; the generation showing the **least**
absolute difference in maze-behavior is shown to be defi-
nitely modified when the tests are made on a multiple-
choice apparatus.

### FERTILITY

Compared with the difficulty of measuring the behavior
tendencies of rats, the measure of fertility is very **simple**
and definite. However, the great amount of time **required**
by the behavior studies prevented the collection of **many**
of the available data on the purely physiological **side.**
As a result of this, instead of the long list of **criteria**
of fertility that have been given by other authors, it is
possible to give only two with any degree of **accuracy**
and completeness. These are: the number of rats in a
litter, and the number of litters. A more detailed **report**
on the data leading to the following conclusions **may be**
found elsewhere (MacDowell, '22*a*).

*Size of Litters.*—A general tendency for the litters of the test rats to be smaller than the controls persists in the summaries of all generations. The difference between the size of the litters from the original treated rats and the litters from the controls is equal to 10.5 per cent. of the size of the control litters. The treated offspring of the treated rats produced litters that were 10.3 per cent. smaller than the litters of their controls. It appears, therefore, that the treatment of the parents of the litters as well as the grandparents does not intensify the reduction in litter size found when only one generation was treated. The untreated offspring from treated rats gave litters that were 11.2 per cent. smaller than their controls, and the untreated offspring from untreated parents and treated grandparents gave litters that were 13.1 per cent. smaller than the controls (see Fig. 4). These differences in individual generations are based on too few cases to be significant when compared with their probable errors, but when the numbers are increased by taking all the generations together, the probable error is reduced so that the difference attains statistical significance (3.6 times its probable error). Litter size, then, gives a result not unlike that given by the behavior data: the tests are inferior in each generation, with no apparent relation to the proximity of the alcohol or the number of generations of treatment.

*Number of Litters.*—Given equal time, the treated pairs produced 0.72 litter per pair while the controls produced 2.07 litters per pair. This is a reduction of $64.8 \pm 3.3$ per cent. in the number of litters, and as it is 19.2 times its probable error, it is significant beyond all question. The test litters were slower in appearing than the controls. The treated rats from treated parents also gave fewer litters than their controls, but instead of a greater reduction than in the previous generation this second treated generation produced relatively more litters. The reduction was $35.4 \pm 6.9$ per cent. of the controls. Coming to the rats not directly treated, the untreated rats

## NUMBERS OF RATS PER LITTER

### AVERAGES IN FOUR GENERATIONS
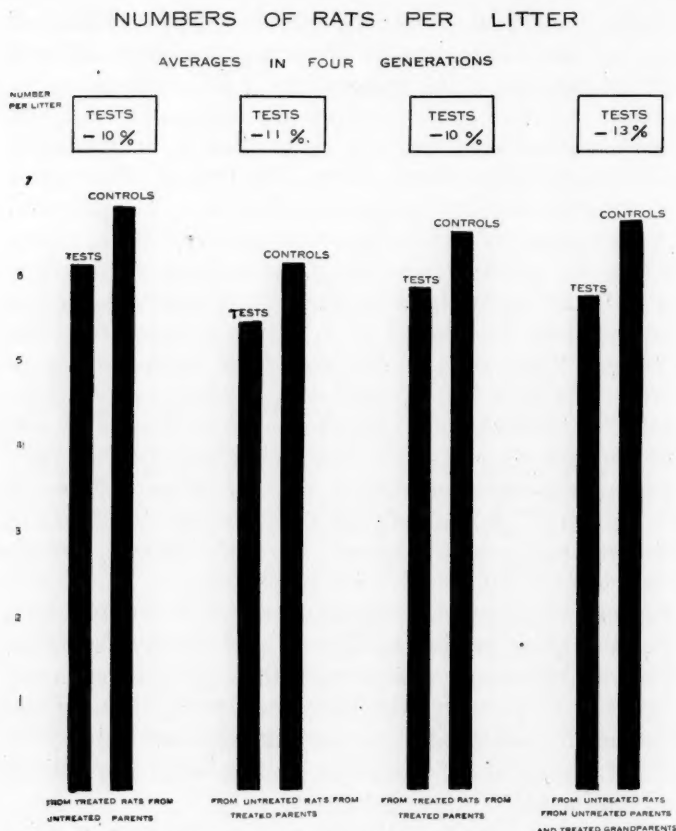


Fig. 4. Average litter size for the controls and tests bearing the relationships to the alcohol treatment indicated.

from treated parents gave $33.3 \pm 8.2$ per cent. *more* litters than their controls, and the untreated rats from untreated parents and treated grandparents produced $55.6 \pm 8.4$ per cent. *more* litters than their controls (see Fig. 5). All of these differences are, without doubt, statistically significant.

*Discussion.*—Two generations of treatment made less difference in number of litters than a single generation of treatment, and two untreated generations following
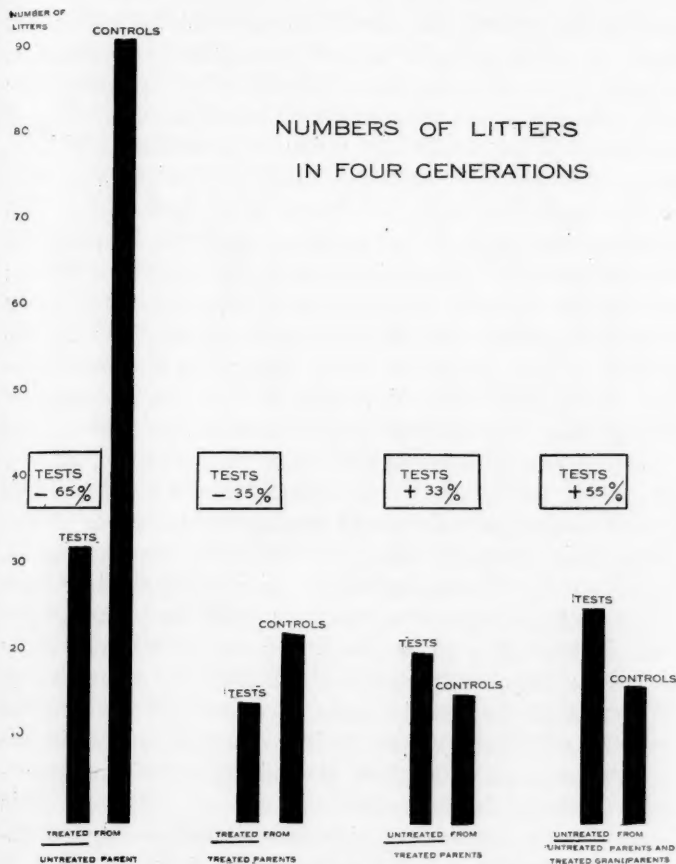
FIG. 5. Relative numbers of litters produced in equal periods by the test and control rats in different generations. Beginning at the left the test litters were produced by the following numbers of pairs: 44, 9, 10, 11; in each case the control litters have been given on the basis of equal numbers of pairs, although the actual numbers of control pairs involved were: 42, 12, 10, 13.

the treatment produced more litters than the controls. The number of litters is strongly reduced when the parents themselves are treated, but when the alcohol is more remote, the reduction vanishes and the untreated descendants of the treated rats produce *more* litters than their controls. To explain the reduction in the number of lit-

ters in the presence of alcohol along purely physiological lines would be a simple matter, but a genetic explanation appears to be required when it comes to the increase over the controls given by untreated descendants of treated animals. No general depression or stimulation will account for the continuation of small litters together with the increase in number of litters in the generations not given alcohol directly. It seems necessary to assume that there are genetic factors influencing the number of litters; alcohol prevents the reproduction of such females as carry factors working in the direction of lower reproductive capacity, so that the litters come alone from females carrying higher litter-producing capacity; the next generation will produce higher numbers of litters than the unselected controls, for the controls still carry all grades of fertility, while the tests lack the genetically lower grades. The treated offspring of treated rats produced fewer litters than their controls, but genetically they were superior, as shown by untreated offspring giving more litters than their controls; they were superior to the first generation, for, instead of a 65 per cent. reduction, they gave only a 35 per cent. reduction in the number of their litters. Whereas the immediate presence of alcohol reduces the number of litters, it acts to increase the number in the next generation; therefore alcohol may produce two results upon a single character in two generations. This could lead to much confusion were it not so easy to understand the first result as the cause of the second.

This selective action of alcohol will account for the results from the number of litters, but will not account for the uniform results given by litter size. If this is a correct statement of the situation, it indicates that the number of litters is influenced by genetic factors that are not identical with those influencing litter size. Although such a distinction between genetic bases for the numbers of litters and litter size has apparently not been made, it is not difficult to conceive, for litter size is largely dependent upon the number and constitution of the germ

cells liberated, while the somatic condition of the mother plays a part in determining whether or not a litter will be produced.  The results from litter size agree strikingly, qualitatively and even quantitatively with those of Stockard and Papanicolaou from similar studies with guinea pigs; the results from the number of litters agree with Pearl's on fowl in so far as they may be interpreted by assuming a selective action of the alcohol working upon existing genetic differences.  In the fowl the alcohol appears to select between germ cells; in the rats it appears to select between mothers of different physiological and genetic grades.

## Weight

The data on weight (see MacDowell, '22*b*) form an extensive series consisting of weekly weighings of practically all the rats raised in the various generations herein described.  Individual growth curves were plotted and from these the weights at six ages were taken for statistical study.  This procedure was necessitated by the fact that all the rats were weighed on the same day each week, so that the rats were of different ages.  The results are based primarily upon the males (see Table II), since the pregnancies of the females make their data less reliable.  However, when the data from the females with arbitrary smoothing of the pregnancy peaks are summarized, the results so obtained support those given by the males.  Each of the four strains shows that the treated rats grew more slowly than the controls.  This is an influence shown by the population as a whole, although there are some individual treated males that remained as heavy as the heaviest controls.  The untreated offspring of the treated rats tended to grow more rapidly than their controls.  This result is not so clear as the opposite result in the preceding generation; the absolute differences are not so large and the strains do not show this in equal measure.  Treated rats from treated parents barely differ at all from their controls.  Very little can be concluded from the weights of the untreated offspring from untreated

## TABLE II

The Weights at Successive Ages of the Various Types of Male Test Rats Compared with their Respective Controls.

| Relation to Alcohol Treatment | Age in Days | Tests Grams Averages | Nos. | Controls Grams Averages | Nos. | Difference | D/P.E. |
|---|---|---|---|---|---|---|---|
| Treated from normal parents | 40 | 70.43 | 70 | 72.95 | 64 | + 2.52 ± 1.40 | 1.8 |
| | 60 | 116.66 | 71 | 129.35 | 65 | +12.69 ± 2.16 | 5.9 |
| | 90 | 149.63 | 61 | 176.63 | 55 | +27.00 ± 3.16 | 8.5 |
| | 120 | 192.10 | 50 | 229.71 | 46 | +37.61 ± 4.07 | 9.2 |
| | 150 | 215.39 | 38 | 257.97 | 39 | +42.58 ± 4.81 | 8.8 |
| | 180 | 235.88 | 34 | 274.34 | 32 | +38.46 ± 5.11 | 7.5 |
| Treated from treated parents | 40 | 83.86 | 15 | 81.81 | 16 | − 2.05 ± 2.73 | 0.7 |
| | 60 | 128.00 | 15 | 131.81 | 16 | + 3.81 ± 3.81 | 1.0 |
| | 90 | 166.50 | 14 | 166.37 | 16 | − 0.13 ± 4.96 | 0.0 |
| | 120 | 198.07 | 14 | 207.50 | 14 | + 9.43 ± 6.01 | 1.5 |
| | 150 | 228.33 | 12 | 236.50 | 14 | + 8.17 ± 6.48 | 1.2 |
| | 180 | 245.70 | 10 | 258.00 | 13 | +12.30 ± 7.44 | 1.6 |
| Untreated from treated parents | 40 | 73.03 | 26 | 63.82 | 29 | − 9.21 ± 2.37 | 3.8 |
| | 60 | 95.53 | 26 | 84.13 | 29 | −11.40 ± 2.56 | 4.4 |
| | 90 | 127.26 | 26 | 117.79 | 29 | − 9.47 ± 3.64 | 2.6 |
| | 120 | 195.26 | 26 | 182.14 | 27 | −13.12 ± 5.13 | 2.5 |
| | 150 | 230.42 | 26 | 219.88 | 27 | −10.54 ± 6.24 | 1.6 |
| | 180 | 249.00 | 25 | 238.85 | 27 | −10.15 ± 7.61 | 1.3 |
| Untreated from untreated parents and treated grandparents | 40 | 75.90 | 10 | 80.57 | 7 | + 4.67 ± 5.08 | 0.9 |
| | 60 | 111.18 | 11 | 97.20 | 10 | −13.98 ± 5.36 | 2.6 |
| | 90 | 131.54 | 11 | 125.90 | 10 | − 5.64 ±11.64 | 0.5 |
| | 120 | 191.63 | 11 | 171.30 | 10 | −20.33 ±16.66 | 1.2 |
| | 150 | 226.09 | 11 | 208.00 | 10 | −18.09 ±16.20 | 1.1 |
| | 180 | 235.54 | 11 | 230.20 | 10 | − 5.34 ±13.52 | 0.4 |

Plus signs indicate the controls heavier; D/P.E.—difference divided by its probable error.

parents and treated grandparents. Two of the three strains represented in this generation show heavier averages for the tests and the third shows heavier averages for the controls; when all the strains together are considered (as in Table II), the test averages are higher at all ages.

This shows a marked similarity to the results from the number of litters; just as the offspring of the treated rats appear to be genetically superior to the controls in the matter of litter production, so they are found to be superior in the matter of weight, with the result that when they themselves are treated, the immediate reducing effect of the alcohol makes them about equal somatically to their controls, instead of growing markedly slower as did their parents. This likeness in results leads to a similar interpretation for the weight as for the number of litters: the alcohol has acted as a selective agent, eliminating germinal material that included factors for slower growth.

## DISCUSSION

In view of the premature termination of these experiments no discussion or interpretation can be justified other than by its possible influence upon future work.

The data on behavior and litter size taken alone may, if the controls are accepted as adequate, be considered to lead to the general interpretation of a direct and definite modification of the germinal material brought about by the alcohol treatment. On the other hand, the data on the number of litters and weight, when taken alone, agree in inviting the interpretation that the alcohol has acted as a selective agent upon germinal differences that were present in the germinal material of the original animals. One tendency pulls the race down, the other, by sacrificing the fullest reproductive expression of the treated individuals, tends to pull it up. The specific conditions found then are end-results that depend upon the interaction of different influences and do not measure directly the amount of influence exerted by the chemical.

Obviously, the situation is complicated, and equally obvious is the impossibility of proving the individual effects of two or more influences acting simultaneously. However, in this case the evidence favoring one supposition (that of selective elimination of germinal material) is very much more convincing than that favoring the supposition of germinal modification. So great, indeed, is this difference that the evidence of direct modification could easily be brushed aside and selective elimination be effectively championed as *the* effect of the alcohol, although even this involves two opposite results depending upon the proximity of the alcohol. But if a true statement of the situation is desired, the conflicting evidence must not be brushed aside.

If the germinal variability existing in the race is greater than the variability caused by the direct action of the alcohol upon the germinal material, the results actually obtained would be expected; that is, the effects of selective elimination would appear more striking in the end results. Since the reductions in litter size and in behavior stand in spite of an apparently much stronger racial improvement, these reductions give stronger support to the supposition that germinal modification is a second activity of the alcohol than is indicated by their magnitude.

The fact that so many different conclusions have been reached by different investigators from experiments with alcohol would in itself suggest very strongly that the action of this chemical upon animals is not simple and direct like the action of an acid upon a base, yet the general attitude toward the problem seems to have been that there should be a single answer, in one direction or the other, and that as soon as an investigator devises the perfect method, this answer will be disclosed. As long as such an attitude persists the alcohol problem will flounder about in the morass of futile and inconclusive papers. The moment chemistry, and later, experimental breeding, turned away from end results to the phenomena

behind them (elements or factors), new epochs were started in these sciences. The problem should not be to judge how bad are the results of alcohol, but rather to find through what channels alcohol may work. The final results will differ in different cases according to differently combined influences of various sorts, just as the same combination of chemicals will yield different results under different conditions, and the same combination of genetic factors will yield various somatic expressions; to know the *modus operandi* of alcohol is fundamental.

### CONCLUSIONS

1. Beginning at the time of weaning, alcohol was administered to white rats every day, in sufficient quantities to cause complete anesthetization. This treatment appears to account for the following differences between the treated rats and their normal sibs:

The treated rats—(*a*) took more time running the maze.

(*b*) produced smaller litters.
(*c*) produced fewer litters.
(*d*) grew more slowly.

2. The treated offspring from the treated rats differed from their controls in the following ways:
The treated offspring—(*a*) tended to take more time in running the maze.

(*b*) produced smaller litters.
(*c*) produced somewhat fewer litters.
(*d*) grew at a very slightly lower rate.

3. The untreated offspring from the treated rats differed from their controls in the following ways:
The untreated offspring—(*a*) took a very little longer in running the maze.

(*b*) produced smaller litters.
(*c*) produced more litters.
(*d*) were heavier.

4. The untreated offspring in the second generation from alcohol treatment differed from their controls in the following ways:

The second generation of untreated offspring—

  (*a*) took more time in running the maze.
  (*b*) produced smaller litters.
  (*c*) produced more litters.
  (*d*) were somewhat heavier.

5. From these results it is concluded that the action of alcohol is complicated; that it works in two or more different ways. The data on behavior and litter size suggest that the alcohol may modify germinal material directly. The data on the number of litters and growth indicate that the direct effect of alcohol upon these characters is in one direction and that its indirect effect is in the opposite direction; this may be interpreted by the assumption of a selective rôle played by the alcohol. It is urged that the alcohol problem can be settled biologically only when, instead of generalizing from the quality of specific end results, we deal with the channels through which alcohol may work.

COLD SPRING HARBOR,
    February, 1922.

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# EXPERIMENTAL STUDIES ON THE DURATION OF LIFE. IV. DATA ON THE INFLUENCE OF DENSITY OF POPULATION ON DURATION OF LIFE IN DROSOPHILA [1]

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## I

FAIRLY early in our experimental work on duration of life in *Drosophila* it became apparent to us that the number of flies per bottle, or, since the bottles used are of uniform size, the density of population, had some influence on the mean duration of life of the flies, when other environmental conditions are constant. Such a relationship might reasonably be expected *a priori,* from what is known of the influence of this factor on human death rates, commonly expressed as Farr's Law (*cf.* Farr, W. (35), Brownlee, J. (36, 37)), and on other biological functions, such as growth (Semper, K. (38), Bilski, F. (39)), resistance to poisons (Drzwina and Bohn (40)), rate of reproduction (Pearl and Surface (41), Pearl and Parker (42)), etc. As soon as it was recognized that this variable, density of population, might influence our experimental results with *Drosophila,* care was taken in setting up experiments to make this a constant in each case. At the same time the records of the earlier work were carefully re-examined to determine what part this variable may have played in the results. Happily it was found that in none of our work so far published upon the duration of life in *Drosophila* had density of population varied enough to have any appreciable effect upon the results or conclusions.

As was recently pointed out by Pearl and Parker (42), however, "there can be no question that this whole matter of influence of density of population, in all senses, upon biological phenomena, deserves a great deal more

investigation than it has had. The indications all are
that it is the most important and significant element in
the biological, as distinguished from the physical, en-
vironment of organisms.'' In pursuance of this idea we
desire to present in this paper our accumulated *statisti-
cal* data on the influence of density of population upon
duration of life in *Drosophila.* This material is to be
regarded as preliminary rather than final. For reasons
which will appear as we proceed, we are inclined to with-
hold final conclusions as to the exact form of the regres-
sion of duration of life upon density until we have com-
pleted an extensive *ad hoc* experimental investigation of
the problem. This experimental work is now in progress
and we hope to be able to report upon it in full in the
course of the next year. In the meantime we have an
impressive body of statistical data gathered from the
control groups of other experiments which it seems de-
sirable to discuss now in a preliminary way.

## II

The data of this study are derived from the normal
control groups of various experiments on duration of
life which we have carried out with *Drosophila,* accord-
ing to the technique described by Pearl and Parker (27).
All of the determinations of duration of life recorded in
the tables of this paper were made under constant condi-
tions of temperature (25° C.), food, etc., as described in
the paper referred to. We have divided the material
for the purposes of the present study into three groups
by stocks (*cf.* Pearl and Parker (27)), *viz.:* (*a*) wild
type flies, including our Old Falmouth, New Falmouth,
and Eagle Point stocks, (*b*) Sepia, and (*c*) Quintuple.

Throughout this paper density of population is taken
as the *initial density* (number of flies per bottle) in the
small bottles used in testing duration of life. Thus a
density of 22 means that 22 flies started in this particular
bottle. As time went on the number was diminished by
deaths until finally none was left. One of course might
use as the variable mean density over the whole life of a

bottle, but a little thought will show that this would be an erroneous procedure when one is dealing with duration of life as the second variable, because *mean* density bears a direct and implicit functional relation to mean duration of life of the flies in the bottle. We shall be on a clearer footing to take *initial* density as the variable. Since the cubical content of the bottles is constant throughout, there is no necessity of reckoning density per c.c. The number of flies per bottle can be taken as the measure of density, and a good deal of useless computation saved.

We are indebted to Dr. John Rice Miner for aid in the computations.

### III

Table I presents the data for the correlation of duration of life with density of population for the wild type flies. The material is in the usual form of a correlation table.

An examination of this surface suggests at once that the regression is probably non-linear. Owing to the manner in which the material was obtained (by compilation of the control series of a number of different experiments) it results that the different arrays have rather highly different total frequencies. The number of flies per bottle was in no way artificially selected or predetermined in this material. Instead it was determined solely by the aggregate fertility of the mating bottles furnishing the material for each particular experiment. As has been explained in the first of these Studies (Pearl and Parker (27)), the routine procedure in our experiments is to put into one bottle for duration of life test all the flies emerging as imagoes at the same time (*i.e.,* usually on the same day). It therefore would result that if the hatch was particularly good on some day, there might be as many as 90 flies in the duration of life bottle initially. On the other hand, there might be only 2 flies, because only that number emerged on that particular day.

Even in spite of the differences in the frequencies of

## TABLE I

### CORRELATION SURFACE FOR THE VARIABLES (a) DURATION OF LIFE, AND (b) INITIAL DENSITY OF POPULATION. WILD STOCKS OF DROSOPHILA

| Age at Death | Number of Flies in Bottle | | | | | | | | | | | | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1- | 5- | 9- | 13- | 17- | 21- | 25- | 29- | 33- | 37- | 41- | 45- | 49- | 53- | 57- | 61- | 65- | 69- | 73- | 77- | 81- | 85- | 89- | 93- | |
| 1- | 15 | 41 | 35 | 29 | 35 | 45 | 37 | 3 | 27 | 4 | 10 | 28 | 20 | | 12 | 19 | | 12 | | | | 2 | | 16 | 390 |
| 7- | 38 | 49 | 47 | 48 | 39 | 38 | 30 | 20 | 22 | 6 | 6 | 8 | 24 | 4 | 13 | 11 | 1 | 17 | 13 | 2 | | | | 43 | 479 |
| 13- | 24 | 55 | 44 | 45 | 59 | 35 | 39 | 20 | 28 | 3 | 3 | 12 | 14 | 23 | 14 | 19 | 1 | 8 | 10 | 5 | 1 | 2 | | 20 | 484 |
| 19- | 19 | 53 | 57 | 30 | 64 | 37 | 38 | 33 | 24 | 17 | 51 | 30 | 32 | 14 | 17 | 33 | 7 | 5 | 46 | 13 | 10 | 22 | | 47 | 699 |
| 25- | 31 | 75 | 59 | 45 | 70 | 42 | 79 | 21 | 31 | 22 | 20 | 20 | 32 | 7 | 17 | 20 | 15 | 7 | 40 | 3 | 15 | 21 | | 5 | 697 |
| 31- | 19 | 87 | 88 | 55 | 76 | 90 | 78 | 47 | 44 | 10 | 47 | 31 | 33 | 5 | 13 | 68 | 11 | 23 | 50 | 13 | 16 | 52 | | 4 | 960 |
| 37- | 14 | 72 | 108 | 84 | 118 | 94 | 80 | 77 | 45 | 19 | 31 | 36 | 60 | 3 | 38 | 70 | 16 | 4 | 20 | 11 | 14 | 27 | | 8 | 1,049 |
| 43- | 21 | 73 | 126 | 86 | 161 | 83 | 80 | 39 | 53 | 16 | 32 | 66 | 67 | | 23 | 18 | 46 | 32 | 33 | 5 | 17 | | | 19 | 1,098 |
| 49- | 19 | 84 | 110 | 109 | 120 | 124 | 117 | 43 | 71 | 20 | 44 | 71 | 70 | | 14 | 35 | 23 | 33 | 7 | 11 | 7 | 2 | | 2 | 1,146 |
| 55- | 10 | 75 | 85 | 102 | 102 | 71 | 70 | 47 | 37 | 38 | 56 | 35 | 37 | | 15 | 38 | 6 | 16 | 1 | 4 | 1 | 12 | | 9 | 910 |
| 61- | 5 | 38 | 61 | 96 | 97 | 55 | 75 | 50 | 39 | 35 | 42 | 55 | 49 | | 11 | 16 | 3 | 18 | 2 | 11 | 1 | 17 | | 13 | 774 |
| 67- | 1 | 25 | 36 | 54 | 66 | 41 | 16 | 19 | 13 | 17 | 18 | 44 | 31 | | 11 | 7 | 3 | 3 | | 1 | | 7 | | | 471 |
| 73- | | 9 | 11 | 33 | 33 | 34 | 9 | 10 | 8 | 13 | 13 | 13 | 15 | | 21 | 9 | 1 | 15 | | 1 | | 5 | | | 278 |
| 79- | | 5 | 4 | 20 | 17 | 13 | 5 | 5 | 4 | 5 | 4 | 10 | 11 | | 2 | 6 | | 6 | | | | | | | 133 |
| 85- | 1 | 2 | 6 | 8 | 3 | 8 | | 3 | 3 | 1 | 2 | 2 | 2 | | 6 | 3 | | 4 | | | | | | | 64 |
| 91- | | | | 3 | | 1 | | | 2 | | 1 | 2 | 1 | | 5 | 2 | | 5 | | | | | | | 28 |
| 97- | | | | | | 1 | | | | | | | | | 1 | | | 1 | | | | | | | 5 |
| 103- | | | | | | | | | | | | | | | 1 | | | | | | | | | | 1 |
| 109- | | | | | | | | | | | | | | | | | | | | | | | | | |
| Total | 217 | 743 | 877 | 847 | 1,060 | 812 | 892 | 456 | 451 | 226 | 380 | 463 | 498 | 56 | 234 | 374 | 133 | 209 | 222 | 80 | 82 | 169 | | 186 | 9,667 |

the several arrays, it still seems probable from mere inspection of the general surface that the regression is non-linear. This idea is strengthened by examination of the regression line itself, shown in Fig. 1.
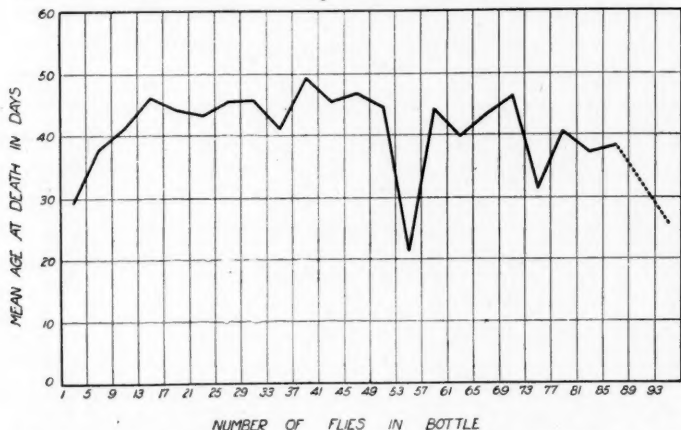


Fig. 1.  Mean duration of life of *Drosophila* for different initial densities of population.  Wild stocks.

It is seen from this diagram that, neglecting the great dip of the line at density 55 which is consequent upon a very small array with large probable error, the general sweep of the curve indicates an optimum density (greatest mean duration of life) in the general region of 35 to 45 flies per bottle, with a decline on either side of that point, but falling lower on the side of high densities than on that of low.

From this table we have the following constants:

$$r = -.0511 \pm .0068,$$
$$\eta = .2443 \pm .0064.$$

There can be no question that the regression is non-linear. Blakeman's (43) criterion has the following value:

$$\zeta = .0571 \pm .0031.$$

It must therefore be concluded that the regression is significantly skew.

The correlation between duration of life and density of population in the case of the Sepia stock is shown in Table II.

## TABLE II

Correlation Surface for the Variables (a) Duration of Life, and (b) Initial Density of Population. Sepia Stock.

| Age at Death | Number of Flies in Bottle | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1– | 5– | 9– | 13– | 17– | 21– | 25– | 29– | 33– | 37– | 41– | 45– | 49– | 53– | 57– | 61– | 65– | 69– | 73– | 77– | 81– | Total |
| 1– | 7 | 29 | 27 | 23 | 13 | 15 | | 1 | 1 | | 2 | | | | | | | | 4 | 1 | 37 | 160 |
| 7– | 10 | 18 | 27 | 27 | 11 | 27 | 2 | 16 | 1 | | 3 | | | | | | | | 3 | 9 | 6 | 168 |
| 13– | 5 | 21 | 23 | 23 | 6 | 33 | 12 | 18 | 1 | 5 | 4 | | | | | | | | 5 | 46 | 1 | 214 |
| 19– | 16 | 27 | 8 | 35 | 9 | 21 | 12 | 21 | 9 | 8 | 8 | | | | 4 | | | | 21 | 17 | 2 | 263 |
| 25– | 7 | 34 | 5 | 20 | 8 | 30 | 17 | 21 | 14 | 35 | 9 | 6 | 10 | | 8 | | | | 38 | 2 | 7 | 301 |
| 31– | 13 | 28 | 40 | 20 | 9 | 20 | 9 | 13 | | 26 | 22 | 30 | 15 | | 6 | | | | 41 | | | 278 |
| 37– | 4 | 28 | 30 | 40 | 15 | 33 | 15 | 7 | 8 | 24 | 11 | 4 | 13 | | 18 | | | | 8 | | | 224 |
| 43– | 9 | 26 | 38 | 35 | 23 | 37 | 12 | 13 | 1 | 15 | 30 | 4 | 3 | | 14 | | | | 27 | 2 | 2 | 294 |
| 49– | 10 | 27 | 36 | 24 | 17 | 19 | 1 | 6 | | 24 | 16 | 1 | 1 | | 6 | | | | 3 | | 15 | 185 |
| 55– | 5 | 18 | 16 | 17 | 19 | 18 | | 2 | | 9 | 8 | 1 | 4 | | 2 | | | | 1 | | 11 | 108 |
| 61– | 3 | 2 | 15 | 10 | 9 | 6 | 1 | 6 | | 2 | 7 | | 1 | | 1 | | | | 1 | | | 67 |
| 67– | | 9 | 6 | 4 | 5 | 3 | | 1 | | 3 | 7 | | 4 | | 1 | | | | | | | 35 |
| 73– | | | 2 | 2 | 2 | 1 | | | | 1 | 2 | | | | | | | | | | | 10 |
| 79– | 1 | | | 2 | | 1 | | | | | 2 | | | | | | | | | | | 6 |
| Total | 90 | 267 | 273 | 282 | 146 | 264 | 81 | 125 | 35 | 152 | 131 | 46 | 51 | | 60 | | | | 152 | 77 | 81 | 2,313 |

Here again there are a number of small arrays and gaps towards the right-hand side of the table, due as before to the method by which the material was got.

The regression of duration of life upon density is shown graphically in Fig. 2.
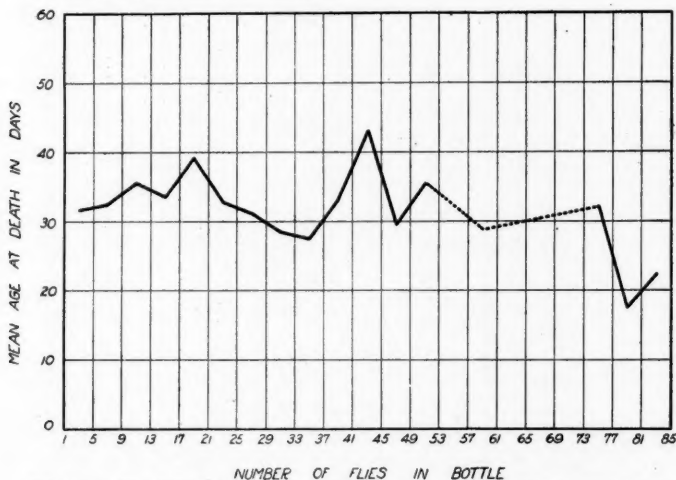


FIG. 2.   Mean duration of life of *Drosophila* for different initial densities of population.   Sepia stock.

It is apparent from inspection here as before that the regression is not clearly linear, but rather indicates an optimum density in the region of 35 to 45 flies per bottle, with a diminished expectation of life at both lower and higher densities.   The constants are

$$r = -.132 \pm .014,$$
$$\eta = .283 \pm .013,$$
$$\zeta = .0629 \pm .0066.$$

The criterion of linearity is nearly 10 times its probable error, and we may therefore conclude for the Sepia stock, as for the wild stocks, that statistically the regression of duration of life upon density of population is significantly skew.

The data for the short-lived Quintuple stock are given in Table III.

Owing to the fact that the Quintuple stock is characterized by low fertility, as well as short duration of life,

## TABLE III

CORRELATION SURFACE FOR THE VARIABLES (*a*) DURATION OF LIFE, AND (*b*) INITIAL DENSITY OF POPULATION.  QUINTUPLE STOCK

| Age at Death | Number of Flies in Bottle | | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1- | 5- | 9- | 13- | 17- | 21- | 25- | 29- | 33- | 37- | 41- | 45- | 49- | 53- | |
| 1–......... | 22 | 18 | 17 | 15 | 9 | 7 | 15 | 1 | ... | ... | ... | ... | ... | 4 | 108 |
| 4–......... | 21 | 33 | 19 | 31 | 4 | 3 | 9 | 11 | ... | ... | ... | ... | ... | 15 | 146 |
| 7–......... | 26 | 70 | 50 | 50 | 13 | 13 | 20 | 4 | ... | ... | ... | ... | ... | 17 | 263 |
| 10–......... | 22 | 38 | 47 | 28 | 19 | 12 | 10 | ... | ... | ... | ... | ... | ... | 7 | 183 |
| 13–......... | 15 | 24 | 28 | 29 | 8 | 13 | 12 | 6 | ... | ... | ... | ... | ... | 3 | 138 |
| 16–......... | 8 | 21 | 20 | 19 | 6 | 7 | 4 | 4 | ... | ... | ... | ... | ... | ... | 89 |
| 19–......... | 11 | 14 | 11 | 12 | 4 | 3 | 1 | ... | ... | ... | ... | ... | ... | 1 | 57 |
| 22–......... | 5 | 13 | 12 | 4 | 6 | 2 | 1 | ... | ... | ... | ... | ... | ... | 1 | 44 |
| 25–......... | 2 | 5 | 13 | 7 | 3 | 3 | 2 | ... | ... | ... | ... | ... | ... | ... | 35 |
| 28–......... | .... | 6 | 3 | 3 | 2 | 2 | 1 | 2 | ... | ... | ... | ... | ... | 2 | 21 |
| 31–......... | .... | 6 | 3 | 1 | ... | 3 | 1 | ... | ... | ... | ... | ... | ... | 1 | 15 |
| 34–......... | 2 | 3 | .... | 3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 8 |
| 37–......... | 1 | 4 | 2 | ... | ... | 1 | ... | ... | ... | ... | ... | ... | ... | 1 | 9 |
| 40–......... | 1 | 3 | 2 | ... | ... | ... | 2 | 1 | ... | ... | ... | ... | ... | 1 | 10 |
| 43–......... | 3 | 2 | 2 | ... | ... | 1 | ... | ... | ... | ... | ... | ... | ... | ... | 8 |
| 46–......... | | | | | | | | | | | | | | | .... |
| 49–......... | .... | 1 | 1 | ... | ... | ... | 1 | ... | ... | ... | ... | ... | ... | ... | 3 |
| Total...... | 139 | 261 | 230 | 202 | 74 | 70 | 79 | 29 | ... | ... | ... | ... | ... | 53 | 1,137 |

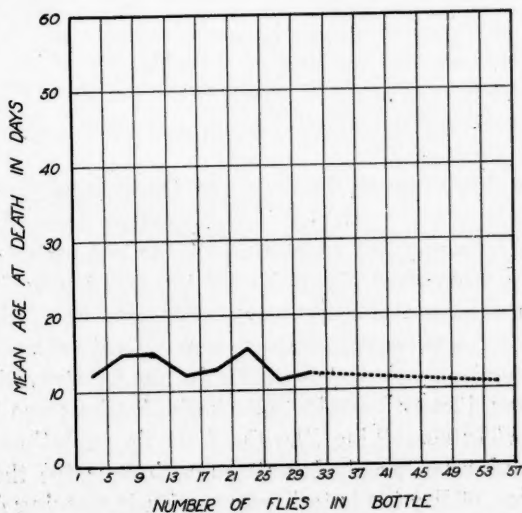this table is less extensive in either direction than the others.



FIG. 3.  Mean duration of life of *Drosophila* for different initial densities of population.   Quintuple stock.

The observed regression line is shown in Fig. 3.

Here the regression appears at once to be substantially linear, and is proved to be by the analytical constants, which are as follows:

$$r = -.057 \pm .020,$$
$$\eta = .120 \pm .020,$$
$$\zeta = .011 \pm .004.$$

The criterion $\zeta$ is less than 3 times its probable error and cannot be regarded as significant.

## IV

Putting all the data together, we have here indisputable evidence that the density of population is a significant factor in influencing the duration of life (or death-rate) in *Drosophila*. The correlation ratio $\eta$ is certainly significant in the case of all three stocks. Its lower value in the case of the Quintuple stock is almost certainly due to the fact that in the Quintuple experience there is not a sufficiently extensive representation of densities. If the other two tables were to be cut off at the density array where the Quintuple is, they also would show a much lower association between the two variables. So, then, the *general portion* of Farr's Law which affirms that death-rate is some function of density of population receives experimental confirmation in a widely different form of life.

When one comes, however, to the precise form discovered by Farr (35) and confirmed by Brownlee (36, 37), the case is not so clear. We do not care to enter upon any detailed discussion of the point now, because we do not care to draw any conclusions as to the true form of the skew regressions observed till we have some additional experimental results in hand. Provisionally, however, it may be said that the indications are that in *Drosophila* something like the following relations hold: (*a*) the lowest density is not the optimum; (*b*) the mean duration of life tends to increase with increasing density up to a certain point which is optimum; (*c*) after the

optimum region has been reached, increasing density is associated with diminished duration of life, which presently falls below the lowest figure found with densities below the optimum. These conclusions must for the present be held as tentative.

## V

In this paper data as to total duration of imaginal life of 13,117 individuals of *Drosophila* are presented in relation to the density of population. It is definitely shown in the case of Wild, Sepia and Quintuple stocks that there is a significant correlation between these variables. The regression of duration of life upon density appears to be significantly skew in the case of Wild and Sepia stocks. The precise form of the regression and theoretical questions connected therewith are left for discussion in a later paper upon the basis of more extensive material.

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(The plan of numbering citations followed is explained in the second of these Studies.)

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# NOTES ON THE HYBRIDS BETWEEN THE CANARY AND TWO AMERICAN FINCHES

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PERHAPS no animal has been so often crossed with other species, and even genera, as the domesticated canary (*Serinus canarius*). Darwin (1885, I, p. 311) speaks of "nine or ten" such crosses, but many more have undoubtedly been made. The hybrids resulting from these crosses are usually, if not always, infertile, and hence are popularly known as "mules." In almost all of these crosses the domesticated canary serves as the female and the wild finch as the male, but bird fanciers occasionally succeed in making the reverse cross. The wild species which is most commonly used for this "mule breeding" is the European goldfinch, *Carduelis carduelis* Linnæus.[1]

This fringillid is one of the handsomest finches in existence, the plumage of the adults of both sexes being made up of a beautiful combination of black, red, white, yellow, and brown patches. The hybrids which result when a yellow, or nearly yellow, canary is crossed with this finch are chiefly interesting for two reasons: (1) because they exhibit an apparently endless chain of variability in coloration, and (2) because their plumage, if dark, is conspicuously streaked, a character which is lacking (as far as external appearance is concerned) in both the yellow canary and the European goldfinch.

Concerning the first of these two points valuable data have been published by Bechstein (1795), Hünefeld (1864), Blakston (1880?), Klatt (1901), Davenport

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[1] According to Chapman (1916, p. 383), this finch was introduced into the United States at Hoboken, N. J. (in 1878), and Boston, and probably still is a resident near both of these places.

(1908), and Galloway (1909). According to these authors, the hybrids between the yellow canary and the European goldfinch may be: (*a*) completely dark, (*b*) mottled (spotted), exhibiting an apparently endless variation in color pattern, or (*c*) entirely white or yellow (very rarely).[2]

The streaking in the dark plumage of canary-European goldfinch hybrids has been variously explained as: (*a*) "derived from the original wild canary" (Darwin, 1885, II, p. 15); (*b*) as reversion to the Serin finch, *Serinus hortulanus* Koch (Klatt, 1901, p. 508); and (*c*) as resulting from the latent streaking (visible in the "green" variety of the domesticated canary) factor of the yellow canary, plus the color factor of the European goldfinch (Davenport, 1908, p. 20).

In 1914 the writer made several attempts to cross the domesticated canary with some of our native American finches, and some of the latter among themselves, since such crosses, if made, seem to have never been recorded. None of these experiments were successful. The work was again taken up in the fall of 1918, and this second attempt yielded several hybrids in 1919 and 1920. For these latter experiments the writer had at his disposal 22 wild finches belonging to the following species: Arkansas goldfinch (*Astragalinus psaltria hesperophilus* Oberholser), willow goldfinch (*Astragalinus tristis salicamans* [Grinnell]),[3] California linnet (*Carpodacus mexicanus frontalis* [Say]), and California purple finch (*Carpodacus purpureus californicus* Baird). Of these 22 wild finches, 5 were reared from eggs placed under

[2] Galloway (1909, p. 4), who has probably reared more canary-finch hybrids than any other breeder, reports the following proportions of self-colored to variegated (mottled) individuals in the case of canary-European gold-finch hybrids: (1) dark plumage (with no white or clear feathers), 172; (2) slightly variegated (a few small white or clear spots in an otherwise dark plumage), 74; (3) variegated (1/4 to 1/2 clear), 75; (4) lightly variegated (1/2 clear to small ticks of dark in an otherwise clear plumage), 19; and (5) completely clear (total absence of dark feathers), 0.

[3] A western sub-species of the American goldfinch (*Astragalinus tristis tristis* Linnæus), popularly known as the "wild canary."

canary females and the remaining 17 were trapped shortly before the breeding season. It is chiefly due to this second fact that the number of hybrids obtained was not larger. All of the experiments were carried out in separate breeding cages. The matings which yielded results were the following:

TABLE I

| Cross No. | Year | ♀ | ♂ | No. of Offspring |
|---|---|---|---|---|
| 1........ | 1919 | Yellow canary × California linnet | | 3 |
| 2........ | 1920 | Yellow canary[4] × Willow goldfinch | | 5 |
| 3........ | 1920 | Willow goldfinch × Arkansas goldfinch | | 4 |

The four hybrids resulting from cross No. 3 (willow goldfinch ♀ × Arkansas goldfinch ♂) died a few days after hatching, and the female could not be induced to breed for a second time. These hybrids differed from ordinary newly-hatched finches and from the eight hybrids obtained from crosses No. 1 and No. 2 in having exceedingly large abdomens, a condition which was probably due to the fact that a large quantity of yolk had not been assimilated.

Cross No. 1 (yellow canary ♀ × California linnet ♂) yielded three hybrids, one of which was accidentally killed when nine days old. During the same summer (1919) Mrs. L. V. Irelan of Berkeley, California, likewise succeeded in rearing a brood (2 males and 2 females) of canary-California linnet hybrids[5] which the writer was able to compare with his own.

Before going into detail regarding the coloration of these canary-California linnet hybrids, it seems desirable to refer briefly to the plumage color of the paternal species, the California linnet. Both sexes of this finch are grayish-brown in color, but, when about three months old, the male turns rose pink, orange red, or scarlet about

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[4] The same female which was used in cross No. 1.

[5] In this case the mother was also completely yellow.

the head, neck, breast and rump. These colors increase in extent and brilliancy with each molt. Males reared and kept in captivity never develop anything but a yellowish-buff color in these regions, and if a mature wild male is confined, its red color, during the molt, likewise becomes yellowish-buff. Both adults and young are conspicuously streaked, especially the latter.

The six[6] canary-California linnet hybrids were all completely dark (self-colored) until the first molt (fall 1919), and closely resembled young California linnets, but their plumage was less intensely dark than that of the latter. During the fall molt of 1919 all of the hybrids became slightly "washed" (tinged) with yellow where the California linnet ♂ is red (or yellowish-buff). This yellow tinge was more conspicuous in the males than in the females and became somewhat more pronounced during the fall molt of 1920.

All six canary-California linnet hybrids are streaked, like the paternal and the "green" variety of the maternal species. As regards size and shape, they differ very little from the parents, both of which are similar in these respects. Their notes are intermediate in timbre between those of the two parental species, the males having a more powerful song than the canary.

In the spring of 1920 the writer paired two of these canary-California linnet hybrids. Both showed an ardent desire to breed and the female exhibited considerable skill in nest building. The first egg was laid on May 6, and several days later a second (May 10). Both of these eggs were only about half the size of canary or California linnet eggs[7] and were dark-blue in color, and not speckled, while those of both parental species are bluish-white and speckled. Both eggs were placed under canary females, but proved to be infertile. The male

[6] The hybrid which was accidentally killed was identical in coloration with these six.

[7] This corroborates similar observations by Bechstein (1795, IV, p. 469) and Blakston (1880?, p. 265), both of whom compare the eggs of canary-finch hybrids with peas.

used in this experiment was also mated with a yellow canary, but, despite much treading, all eggs were clear.

From cross No. 2 (yellow canary ♀ × willow goldfinch ♂) five[8] hybrids were obtained. A few years before, Dr. H. C. Bryant of the California Fish and Game Commission also succeeded in rearing a canary-willow goldfinch hybrid, concerning which he has been kind enough to furnish the writer with complete information.

Before considering the plumage color of these canary-willow goldfinch hybrids, it seems again desirable to sketch briefly that of the wild finch: Both young and adults of the willow goldfinch are chiefly olive-brown and black in color, but the sexually mature male turns canary-yellow during the summer, with the exception of the wings, tail and a small patch on the head, which remain black. Neither young nor adults show any streaking.[9]

The three canary-willow goldfinch hybrids reared by the writer are (January 6th, 1921) colored as follows: No. 1, completely dark (self-colored); No. 2, likewise, except for a few yellow feathers near the left eye; No. 3, dark, with a yellow band, about 5 mm. in width, running across the head; No. 4 (reared by Dr. Bryant),[10] dark, with some white feathers on the tail. All of the hybrids reared by the writer are conspicuously streaked, which, according to Dr. Bryant, was also true of hybrid No. 4.

As regards size and shape, the writer's canary-willow goldfinch hybrids closely resemble the canary (this was also true of hybrid No. 4), especially in shape of beak and length of tail, in which respects there is a considerable difference between the two parental species. As in

[8] Two of these died shortly after hatching and hence furnished no reliable data as regards coloration.

[9] This is also true of the remaining North American members of the genus Astragalinus, the Arkansas and the Lawrence goldfinch (*Astragalinus lawrencei* Cassin), except that in the case of the latter, the lower parts of the young are indistinctly streaked (cf. Bailey, 1912, pp. 322, 323).

[10] The canary mother of this hybrid was also completely yellow.

the case of cross No. 1 (yellow canary ♀ × California linnet ♂), the notes of the hybrids are intermediate in timbre between those of the parents.

We now come to the question as to how these hybrids compare with other canary-finch hybrids, and in how far they conform with Mendel's laws of inheritance. It will be noticed that in the case of the canary-California linnet hybrids, as in many mammalian crosses, dark color is completely dominant over light color, but the number of offspring (7) is too small to warrant the conclusion that this will always prove to be the case. On the other hand, as regards the canary-willow goldfinch hybrids, there is no complete dominance of one color, the hybrids in this case showing a similar variability to that of canary-European goldfinch hybrids.

Davenport (1908, p. 23) believes that the variability in plumage color of canary-finch hybrids is entirely due to the "mottling factor" of the yellow canary. He says (p. 23):

It [the yellow canary] carries a mottling factor. Consequently when the yellow canary is crossed with a pigmented canary or with a finch the hybrids are mottled.

In support of this hypothesis he makes the following statement:

That it is the yellow canary which contains the mottling factor and is the source of the variability of the hybrids is shown by the fact that (1) hybrids with the green canary do not vary in this fashion, and (2) hybrids between any two species of finches—of which many are bred by fanciers—are " cast in one mold."

As regards the first of these two points, it may be said that one should not expect canary-finch hybrids from a "green" (self-colored) canary to show yellow markings as frequently as when a yellow canary is used. In regard to the second point, Davenport (1908) seems to have overlooked the fact that Blakston (1880?), on whose authority this statement was probably based, states only (p. 274) that all bullfinch-goldfinch "mules" are "cast in one mould." In fact one of Blakston's (1880?) re-

marks clearly indicates that this is not true of the hybrids between all species of finches, for on the next page (275) he makes the following statement concerning the "much more common" greenfinch-goldfinch hybrid:

It is not a very pretty bird, . . . partaking to a considerable extent of its [the greenfinch's] dull colour, though occasionally a more brilliant example than usual, having a good deal of the Goldfinch character about it, appears on the stage.

Davenport's (1908) conclusion therefore does not seem to be very well founded.

Results published by Galloway (1909) since the appearance of Davenport's (1908) paper seem to throw some light on this question. As already stated, this author (Galloway) obtained 172 dark (self-colored) to 168 variegated (mottled) offspring from his canary-European goldfinch (*Carduelis carduelis*) crosses. However, when he used the siskin (*Carduelis spinus*), a closely related but darker species, he obtained nearly three times as many (36 to 13) self-colored as mottled individuals, that is, almost a 3 to 1, instead of a 1 to 1 ratio. These results, supported by those set forth in this paper, suggest that the frequency of mottling in canary-finch hybrids is not solely due to the yellow canary,[11] but probably also depends on the coloration of the wild finch.

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[11] A similar problem exists in regard to the mottled seed-coat of the $F_1$ of certain pigmented-white bean crosses. Shull (1907) suggested that it is the white, and not the pigmented bean to which the mottling is due. However, Tschermak (1904, 1912) has shown that in some cases it is the pigmented bean which is the source of the mottling, a view which was later accepted by Shull (1908, pp. 437–439).

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# COEFFICIENTS OF INBREEDING AND RELATIONSHIP

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IN the breeding of domestic animals consanguineous matings are frequently made. Occasionally matings are made between very close relatives—sire and daughter, brother and sister, etc.—but as a rule such close inbreeding is avoided and there is instead an attempt to concentrate the blood of some noteworthy individual by what is known as line breeding. No regular system of mating such as might be followed with laboratory animals is practicable as a rule.

The importance of having a coefficient by means of which the degree of inbreeding may be expressed has been brought out by Pearl[1] in a number of papers published between 1913 and 1917. His coefficient is based on the smaller number of ancestors in each generation back of an inbred individual, as compared with the maximum possible number. A separate coefficient is obtained for each generation by the formula

$$Z_n = 100 \ (1 - \frac{q_{n+1}}{p_{n+1}}) = 100 \ (1 - \frac{q_{n+1}}{2^{n+1}})$$

where $q_{n+1}/2^{n+1}$ is the ratio of actual to maximum possible ancestors in the $n + $ 1st generation. By finding the ratio of a summation of these coefficients to a similar summation for the maximum possible inbreeding in higher animals, viz., brother-sister mating, he obtains a single coefficient for the whole pedigree.

This coefficient has the defect, as Pearl himself pointed

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[1] AMERICAN NATURALIST, 1917, 51: 545–559; 51: 636–639.

out, that it may come out the same for systems of breeding which we know are radically different as far as the effects of inbreeding are concerned. For example, in the continuous mating of double first cousins, an individual has two parents, four grandparents, four great grandparents and four in every generation, back to the beginning of the system. Exactly the same is true of an individual produced by crossing different lines, in each of which brother-sister mating has been followed. Yet in the first the individual will be homozygous in all factors if the system has been in progress sufficiently long; in the second he will be heterozygous in a maximum number of respects.

In order to overcome this objection Pearl has devised a partial inbreeding index which is intended to express the percentage of the inbreeding which is due to relationship between the sire and dam, inbreeding being measured as above described. A coefficient of relationship is used in this connection. These coefficients have been discussed by Ellinger[2] who suggests certain alterations and extensions by means of which the total inbreeding coefficient, a total relationship coefficient and a total relationship-inbreeding index for a given pedigree can be compared on the same scale.

An inbreeding coefficient to be of most value should measure as directly as possible the effects to be expected on the average from the system of mating in the given pedigree.

There are two classes of effects which are ascribed to inbreeding: First, a decline in all elements of vigor, as weight, fertility, vitality, etc., and second, an increase in uniformity within the inbred stock, correlated with which is an increase in prepotency in outside crosses. Both of these kinds of effects have ample experimental support as average (not necessarily unavoidable) consequences of inbreeding. The best explanation of the decrease in vigor is dependent on the view that Mendelian

[2] AMERICAN NATURALIST, 1920, 54: 540–545.

factors unfavorable to vigor in any respect are more
frequently recessive than dominant, a situation which is
the logical consequence of the two propositions that
mutations are more likely to injure than improve the
complex adjustments within an organism and that injuri-
ous dominant mutations will be relatively promptly
weeded out, leaving the recessive ones to accumulate,
especially if they happen to be linked with favorable
dominant factors. On this view it may readily be shown
that the decrease in vigor on starting inbreeding in a
previously random-bred stock should be directly pro-
portional to the increase in the percentage of homozygo-
sis. Numerous experiments with plants and lower
animals are in harmony with this view. Extensive ex-
periments with guinea-pigs conducted by the Bureau of
Animal Industry are in close quantitative agreement.
As for the other effects of inbreeding, fixation of char-
acters and increased prepotency, these are of course in
direct proportion to the percentage of homozygosis.
Thus, if we can calculate the percentage of homozygosis
which would follow on the average from a given system
of mating, we can at once form the most natural coeffi-
cient of inbreeding. The writer[3] has recently pointed out
a method of calculating this percentage of homozygosis
which is applicable to the irregular systems of mating
found in actual pedigrees as well as to regular systems.
This method, it may be said, gives results widely different
from Pearl's coefficient, in many cases even as regards
the relative degree of inbreeding of two animals.

Taking the typical case in which there are an equal
number of dominant and recessive genes ($A$ and $a$) in
the population, the random-bred stock will be composed
of 25 per cent. $AA$, 50 per cent. $Aa$ and 25 per cent. $aa$.
Close inbreeding will tend to convert the proportions to
50 per cent. $AA$, 50 per cent. $aa$, a change from 50 per
cent. homozygosis to 100 per cent. homozygosis. For a
natural coefficient of inbreeding, we want a scale which

[3] *Genetics*, 1921, 6: 111–178.

runs from 0 to 1, while the percentage of homozygosis is running from 50 per cent. to 100 per cent. The formula $2h-1$, where $h$ is the proportion of complete homozygosis, gives the required value. This can also be written $1-2p$ where $p$ is the proportion of heterozygosis. In the above-mentioned paper it was shown that the coefficient of correlation between uniting egg and sperm is expressed by this same formula, $f = 1-2p$. We can thus obtain the coefficient of inbreeding $f_b$ for a given individual $B$, by the use of the methods there outlined.

The symbol $r_{bc}$, for the coefficient of the correlation between $B$ and $C$, may be used as a coefficient of relationship. It has the value 0 in the case of two random individuals, .50 for brothers in a random stock and approaches 1.00 for individuals belonging to a closely inbred subline of the general population.

In the general case in which dominants and recessives are not equally numerous, the composition of the random-bred stock is of the form $x^2\ AA$, $2xy\ Aa$, $y^2\ aa$. The percentage of homozygosis is here greater than 50 per cent. The rate of increase, however, under a given system of mating, is always exactly proportional to that in the case of equality. The coefficient is thus of general application.

If an individual is inbred, his sire and dam are connected in the pedigree by lines of descent from a common ancestor or ancestors. The coefficient of inbreeding is obtained by a summation of coefficients for every line by which the parents are connected, each line tracing back from the sire to a common ancestor and thence forward to the dam, and passing through no individual more than once. The same ancestor may of course be involved in more than one line.

The path coefficient, for the path, sire $(S)$ to offspring $(O)$, is given by the formula $p_{o.s} = \frac{1}{2}\sqrt{(1 + f_s)/(1 + f_o)}$, where $f_s$ and $f_o$ are the coefficients of inbreeding for sire

and offspring, respectively. The coefficient for the path, dam to offspring, is similar.

In the case of sire's sire $(G)$ and individual, we have $p_{o.g} = p_{o.s}\ p_{s.g} = \frac{1}{4}\sqrt{(1 + f_g)/(1 + f_o)}$, and for any ancestor $(A)$ we have for the coefficient pertaining to a given line of descent $p_{o.a} = (\frac{1}{2})^n \sqrt{(1 + f_a)/(1 + f_o)}$, where $n$ is the number of generations between them in this line.

The correlation between two individuals $(r_{bc})$ is obtained by a summation of the coefficients for all connecting paths.

Thus

$$r_{bc} = \Sigma p_{ba} p_{ca}$$
$$= \Sigma \left(\frac{1}{2}\right)^{n+n'} \frac{1 + b_a}{\sqrt{(1 + b_b)(1 + b_c)}},$$

where $n$ and $n'$ are the number of generations in the paths from $A$ to $B$ and from $A$ to $C$, respectively.

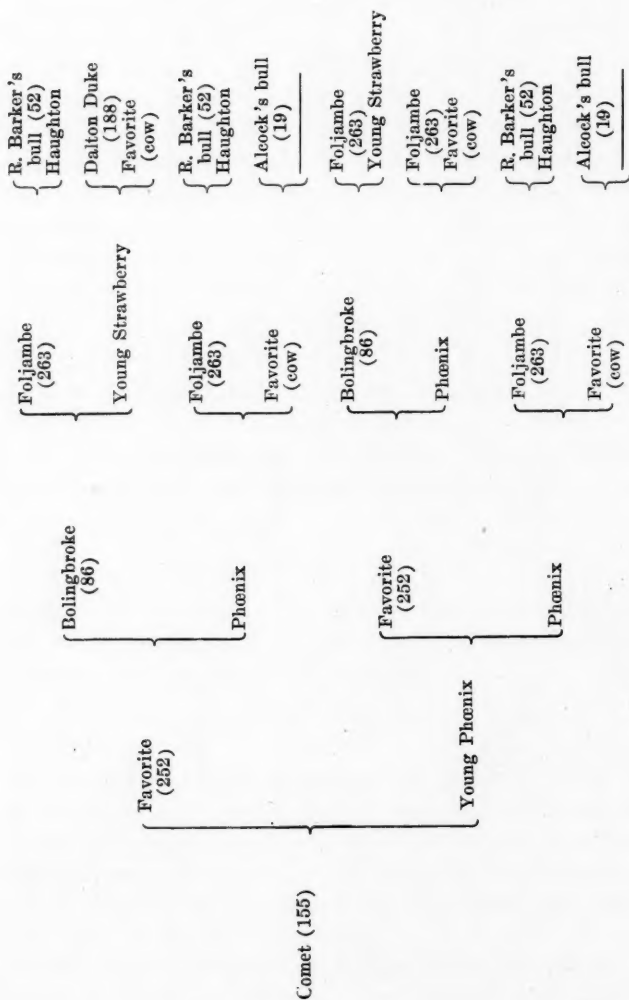The formula for the correlation between uniting gametes, which is also the required coefficient of inbreeding, is

$$f_o = \frac{1}{2}r_{sd}\sqrt{(1 + f_s)(1 + f_d)},$$

where $r_{sd}$ is the correlation between sire and dam and $f_s$ and $f_d$ are coefficients of inbreeding of sire and dam. Substituting the value of $r_{sd}$ we obtain

$$f_o = \Sigma (\tfrac{1}{2})^{n+n'+1}(1 + f_a).$$

If the ancestor $(A)$ is not inbred, the component for the given path is simply $(\frac{1}{2})^{n+n'+1}$ where $n$ and $n'$ are the number of generations from sire and dam respectively to the ancestor in question. If the common ancestor is inbred himself, his coefficient of inbreeding $(f_a)$ must be worked out from his pedigree.

This formula gives the departure from the amount of homozygosis under random mating toward complete homozygosis. The percentage of homozygosis (assuming 50 per cent. under random mating) is $\frac{1}{2}(1 + f_o)$ $\times 100$.

Roan Gauntlet 45276 (35284)

Royal Duke of Gloster 20901 (29864)

Grand Duke of Gloster 19900 (26288)

Champion of England (17526)

Lancaster Comet (11663)
Virtue

Lord Raglan (13244)
Duchess of Gloster 6th

Duchess of Gloster 9th

Mimulus

Champion of England (17526)

Lancaster Comet (11663)
Virtue

Lord Raglan (13244)
Maidstone

Mistletoe

Princess Royal

Champion of England (17526)

Lancaster Comet (11663)

The Queens Roan (7389)

Virtue

Plantagenet (11906)
Verdant

Carmine

The Czar (20947)

Lord Raglan (13244)
Corianda

Cressida

John Bull (11618)
Clipper

Comet (155)

Favorite (252)

Young Phœnix

Bolingbroke (86)

Phœnix

Favorite (252)

Phœnix

Foljambe (263)

Young Strawberry

Foljambe (263)

Favorite (cow)

Bolingbroke (86)

Phœnix

Foljambe (263)

Favorite (cow)

R. Barker's bull (52) Haughton

Dalton Duke (188)

Favorite (cow)

R. Barker's bull (52) Haughton

Alcock's bull (19)

Foljambe (263)

Young Strawberry

Foljambe (263)

Favorite (cow)

R. Barker's bull (52) Haughton

Alcock's bull (19)

By this means the inbreeding in an actual pedigree, however irregular the system of mating, can be compared accurately with that under any regular system of mating.

As an illustration, take the pedigree of Roan Gauntlet, a famous Shorthorn sire, bred by Amos Cruickshank. This bull traces back in every line to a mating of Champion of England with a daughter or granddaughter of Lord Raglan. For the present purpose we will assume that these bulls were not at all inbred themselves and not related to each other. Since the sire traces twice to Champion of England and twice to Lord Raglan and the dam once to each bull, there are in all four lines by which the sire and dam are connected.

| Individual | Common Ancestors of Sire and Dam | $f_a$ | $n$ | $n'$ | $(\tfrac{1}{2})^{n+n'+1}$ $\times (1 + b_a)$ |
|---|---|---|---|---|---|
| Roan Gauntlet 45,276 (35,284) | Champion of England (17,526)............ | 0 | 2 | 1 | .062500 |
| | | | 2 | | .062500 |
| | Lord Raglan (13,244).. | 0 | 3 | 3 | .007812 |
| | | | 3 | | .007813 |
| | | | | | .140625 |

The coefficient of inbreeding comes out 14.1 per cent., a rather low figure when compared to such systems as brother-sister mating (one generation 25 per cent., two generations 37.5 per cent., three generations 50 per cent., ten generations 88.6 per cent.) or parent-offspring mating, (one generation 25 per cent., two generations 37.5 per cent., three generations 43.8 per cent., approaching 50 per cent. as a limit).

As an example of closer inbreeding, take the pedigree of Charles Collings' bull, Comet. The sire was the bull Favorite and the dam was from a mating of Favorite with his own dam. As Favorite was himself inbred to some extent, it is necessary to calculate first his own coefficient of inbreeding.

| Individual | Common Ancestors of Sire and Dam | $f_a$ | $n$ | $n'$ | $(\tfrac{1}{2})^{n+n'+1}$ $\times (1 + f_a)$ |
|---|---|---|---|---|---|
| Favorite (252) | Foljambe (263)........ | 0 | 1 | 1 | .1250 |
|  | Favorite (cow)........ | 0 | 2 | 1 | .0625 |
|  |  |  |  |  | .1875 |
| Comet (115) | Favorite (252)........ | .1875 | 0 | 1 | .2969 |
|  | Phœnix.............. | 0 | 1 | 1 | .1250 |
|  | Foljambe............ | 0 | 2 | 2 | .0312 |
|  | Favorite (cow)........ | 0 | 3 | 2 | .0156 |
|  |  |  |  |  | .4687 |

In the case of Comet, Foljambe and Favorite (cow) each appears twice in the pedigree of the sire and three times in the pedigree of the dam. However, only those pedigree paths which connect sire and dam and which do not pass through the same animal twice are counted. The listing of Favorite (252) and Phœnix as common ancestors eliminates all but one path in each case as regards Foljambe and Favorite cow. The remaining paths are those due to the common descent of Bolingbroke, the sire's sire and Phœnix as the dam's dam from the above two animals.

By tracing the pedigrees back to the beginning of the herd book, the coefficients of inbreeding are slightly increased. This meant going back to the seventh generation for one common ancestor of the sire and dam of Favorite. The coefficient in the case of Favorite becomes .192 instead of .188 and that of Comet .471 instead of .469. Remote common ancestors in general have little effect on the coefficient. It will be noticed that Comet has a degree of inbreeding almost equal to three generations of brother-sister mating or an indefinite amount of sire-daughter mating where the sire is not himself inbred.

# THE ASSORTMENT OF CHROMOSOMES
## IN TRIPLOID DATURAS

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The present article is the one of a number of proposed papers which will deal with the behavior of the chromosomes in the different classes of Datura mutants, the correlation of the chromosomal differences with changes in structural and other characters, and with the ratios in which Mendelian allelomorphs are found in the offspring. The method mainly used in the microscopical examination, and the general principles involved, are given in two papers already in press for THE AMERICAN NATURALIST.

*Sizes of Chromosomes.*—The diploid *Datura Stra-*
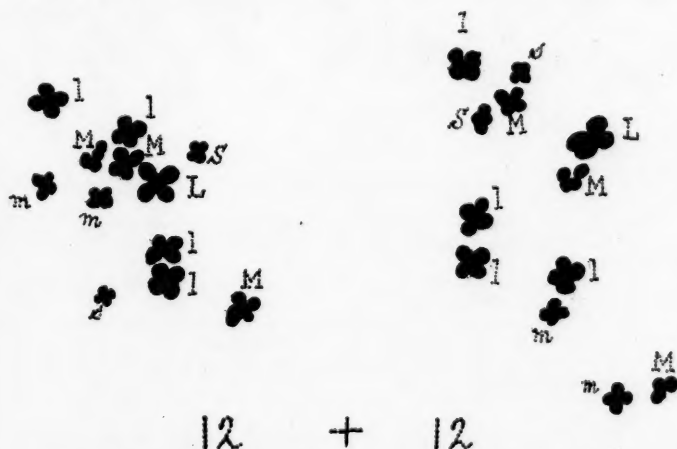


FIG. 1. Second metaphases of a normal Datura in a pollen-mother-cell. The chromosomes are about to divide into two, and each half is constricted. (This figure, as well as Figs. 2 to 6, is a camera drawing of a preparation in iron-aceto-carmine, the cytoplasm having been thinned and flattened by appropriate pressure so that the chromosomes were in optical contact with the cover-glass.)

339

*monium* shows, in the metaphase of the second division in the pollen-mother-cells (Fig. 1), two groups, each consisting of 1 extra large chromosome, 4 large, 3 large medium, and 2 small medium chromosomes, 1 small and 1 extra small chromosome. Thus the somatic formula is $2(L+$
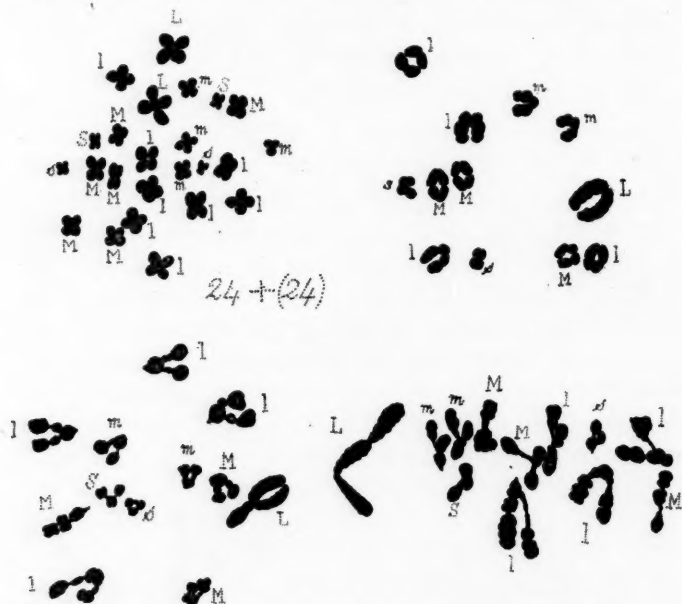


Fig. 2. One second metaphase plate of a tetraploid Datura, in a pollen-mother-cell.

Fig. 3. Late prophase of a normal Datura in a pollen-mother-cell. The size differences are especially distinct, for the smaller chromosomes have condensed earlier.

Fig. 4. Late prophase of a triploid Datura. The largest chromosome set (trivalent) was the latest to condense.

Fig. 5. Late prophase of a triploid Datura. The largest chromosome set is hook-shaped. (The late prophase or early metaphase trivalents often have the form of a ring with a handle, which is indicated in only one trivalent in Fig 4, and is not shown in Fig. 5.)

$4l + 3M + 2m + S + s)$. Tetraploid plants have arisen, in rare cases, from these diploid Daturas (2). They show (Fig. 2) twice as many chromosomes in each of the size classes, and have the somatic formula $4(L + 4l + 3M + 2m + S + s)$. Out of many crosses of tetraploid

Daturas by pollen from normals, 4 triploid plants have resulted (3). Their somatic formula is shown to be $3(L + 4l + 3M + 2m + S + s)$. Similar results have been obtained for triploid hyacinths by de Mol (7).

*Attraction of Homologous Chromosomes.*—In the normal Daturas the late prophase or early metaphase of the first division in the pollen-mother-cells shows 12 sets with two united chromosomes (bivalents) in each (Fig. 3). These bivalents can readily be arranged in the six size classes. In the corresponding stage of the triploid Daturas there are 12 sets of three united chromosomes each, and these trivalents can be arranged according to the size formula (Fig. 4). Sometimes two of the three rod-shaped chromosomes are united together at both ends, and the third is joined on at one end only, or the three may form a hook (Fig. 5). Some trivalents were seen by Osawa in triploid mulberries (8), and a group of 9 trivalents was also found in a triploid Canna (1). (The complete group of 9 trivalents has also been seen in 4 other triploid Cannas.)
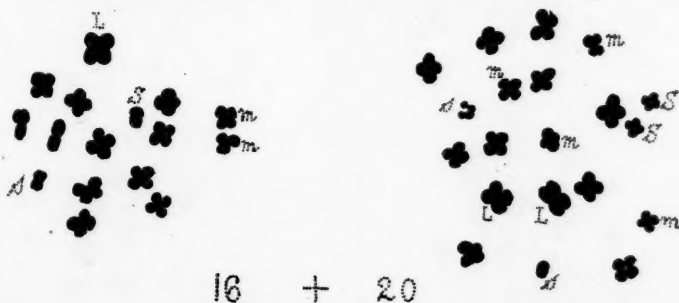


FIG. 6. Second metaphases in a pollen-mother-cell of a triploid Datura. The large and large medium chromosomes were not separable in this preparation.

*Separation (Disjunction) of Chromosomes.*—So far as seen in Datura, two chromosomes usually pass to one pole, and one chromosome to the other, from each trivalent, as is the case in triploid Cannas (1).

*Assortment of Chromosomes.*—From one triploid

plant both groups of chromosomes were counted in each
of 84 pollen-mother-cells, which were in the second meta-
phases, and showed no detached chromosomes (Fig. 6).
The assortments are given in Table I.

TABLE I

ASSORTMENT OF CHROMOSOMES IN 84 POLLEN-MOTHER CELLS OF TRIPLOID
DATURA, 19729(1)

*Metaphase of Second Division*

| Assortment of Chromosomes | 12 + 24 | 13 + 23 | 14 + 22 | 15 + 21 | 16 + 20 | 17 + 19 | 18 + 18 |
|---|---|---|---|---|---|---|---|
| Nos. of double groups....... | 1 | 1 | 6 | 13 | 17 | 26 | 20 |
| Calculated on random orientation of trivalents......... | 0.04 | 0.5 | 2.7 | 9.0 | 20.3 | 32.5 | 19.0 |



FIG. 7.   Early anaphase of the second division in a pollen-mother-cell of a
triploid Datura.   (The upper right-hand plate was shifted upwards in drawing.)
One of the 3 extra large chromosomes had apparently been detached at the first
anaphase, and divided at the second division.   Probably a tetrad with 2 micro-
cytes would have resulted.

It is evident that the orientation of the trivalents in the first metaphase must be nearly or quite a random one, as was suggested in triploid Œnotheras (5, 6) and mulberries (8), and as is the case in triploid Cannas (1). (Nearly similar results were also obtained from a total of 58 single-metaphase plates from this triploid Datura.)

*Detachment of Chromosomes.*—Three buds yielded 62 pollen-mother-cells with both second-metaphase plates countable, and among these there were six cells showing that one chromosome had been detached at the first anaphase (Fig. 7), one cell showing detachment of two chromosomes, and one cell showing both one and two detached chromosomes. Thus there were about 13 per cent. of cases of detachment. These detached chromosomes (8) form microcytes when the pollen-mother-cells constrict to form tetrads (Fig. 8). Table II shows the num-
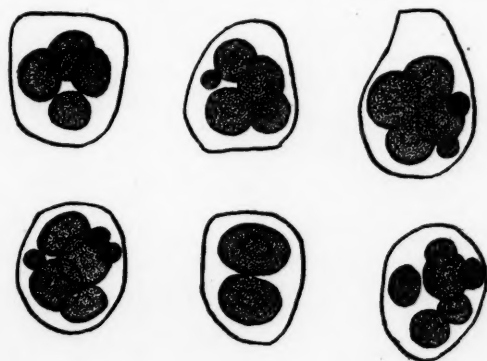


Fig. 8.   Tetrads, etc., of a triploid Datura.   Above: (1) a normal tetrad; (2) a tetrad with one microcyte; (3) a tetrad with 2 microcytes.   Below: (1) a tetrad with 4 microcytes; (2) two giant cells; (3) rare form with 6 not very unequal cells.

bers of microcytes seen in nearly 3,500 tetrads from 3 triploid plants. The average is 13 per cent. of cases of detachment, but the variation in different buds appears too great to be due to chance alone. In 100 pollen-grains there would be about 5 microcytes.

*Non-reduction.*—In belated pollen-mother-cells the chromosomes in the trivalents assume the four-lobed condition of those in the adjoining cells which are in the metaphase of the second division. The first nuclear divi-

TABLE II

DETACHMENT OF CHROMOSOMES. NON-REDUCTION

*Pollen Tetrads of Triploid Plants. (Percentages)*

| Nos. of Microspores...... | Regular Microspores | | | | | Double-sized Microspores | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | 4 | 4 | 4 | 4 | 2 | 2 | 2 | Etc. | Percentage of Cases of Detachment | Nos. of Tetrads |
| Nos. of Microcytes....... | — | 1 | 2 | 3 | 4 | — | 1 | 2 | | | |
| Plant and Bud | | | | | | | | | | | |
| 19729 (1) *a*.. | 67.0 | 20.0 | 9.0 | 0.7 | 0.5 | 2.0 | 0.5 | 0.2 | | 30.9 | 403 |
| 19729 (1) *b*.. | 91.5 | 3.0 | 5.3 | 0.2 | | | | | | 8.5 | 436 |
| 19729 (1) *c*.. | 90.3 | 3.3 | 5.9 | 0.2 | 0.2 | | | | | 9.6 | 425 |
| 19729 (1) *d*.. | 96.1 | 1.6 | 0.9 | — | — | 1.4 | | | | 2.5 | 433 |
| 20345 (1) *a*.. | 83.8 | 7.9 | 8.1 | 0.2 | | | | | | 16.2 | 444 |
| 20345 (1) *b*.. | 97.8 | 0.5 | 0.7 | — | — | 0.7 | | | 0.2 | 1.2 | 412 |
| 20345 (1) *c*.. | 98.0 | 0.8 | 0.3 | — | — | 1.0 | | | | 1.1 | 400 |
| 20380 (1) −.. | 65.5 | 19.0 | 14.6 | — | — | 0.6 | — | 0.4 | | 34.0 | 542 |
| Average..... | 86.3 | 7.0 | 5.6 | 0.2 | 0.1 | 0.7 | 0.1 | 0.1 | 0.03 | 13.0 | Total 3,495 |

| Microcytes to 100 pollen-grains = 4.9 | Percentage of double-sized pollen-grains = 0.4 |
|---|---|

sion is entirely omitted, there is no reduction (8), and two nuclei with 36 chromosomes each are formed at the second division. The two cells which result are twice the size of the average microspores, and can be seen in the pollen as giant grains. Non-reduction may be greatly increased by transient cold. It averaged 0.4 per cent. in the tetrads. A hundred full pollen-grains were measured at random from each of 8 flowers on 4 triploid plants. The average was 0.5 per cent. of giant grains.

*Chromosomes of Functional Egg-cells.*—In one triploid Datura, from three (or fewer) capsules pollinated by a normal, there were produced 75 mature plants, 67 of which had their chromosomes counted.

## TABLE III

CHROMOSOMES OF PROGENY OF TRIPLOID DATURA POLLINATED BY DIPLOID

| Nos. and Assortment of Chromosomes | 12 + 12 | 13 + 12 | 14 + 12 or 13 + 13 | ........ | 24 + 12 .. 18 + 18 |
|---|---|---|---|---|---|
| Nos. of plants | 24 | 33 | 10 | ........ | ........ |
| Calculated on random assortment for 4096 ovules. | 1 | 12 | 66 | etc. | ........ |

The number of normal progeny shown in Table III is much beyond expectation (on the hypothesis that orientation of trivalents in the first division of the megaspore mother-cell is random), even if we allow the excessive total of over 4,000 ovules to 3 capsules. Detachment of chromosomes in the megaspore-mother-cells to the maximum extent found in the pollen-mother-cells will only partially account for this excess. Similar results were obtained by van Overeem with triploid *Œnothera biennis* pollinated by the normal (9).

*Triploid Inheritance.*—The 75 progeny showed triploid or trisomic (not disomic) inheritance (2) of two probably independent pairs of genes, those for purple and white flowers, and those for prickly and smooth capsules.

*Distribution of Extra Chromosomes.*—Among the 33 plants with one extra chromosome, cases were found where this extra chromosome was extra large, large, medium, small, or extra small. These plants showed 11 bivalents and 1 trivalent at the late prophase and early first metaphase. Ten different forms were recognized by external features among 30 of the 33 forms with an extra chromosome. (Three plants have not yet been identified.) Among these ten forms, 1 form (Globe) occurred 5 times, 3 forms (Buckling, Ilex, and Reduced) occurred 4 times, 2 forms (Glossy and Elongate) occurred 3 times, 3 forms (Rolled, Cocklebur, and Poinsettia) occurred twice, and 1 form (Microcarpic) occurred once. The expectations for each of 12 possible forms are presumably equal, namely 2.5. The Datura plants with 2 extra

chromosomes so far examined showed 10 bivalents and 2 trivalents at the first prophase.

Thus the random assortment of chromosomes in triploid Daturas parallels the conclusions as to the random assortment of genes in triploid (trisomic) inheritance, and adds to the evidence for the chromosomal theory of heredity given by the cytological and genetic work on Drosophila (4) and other insects.

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# ŒSTRUS AND FECUNDITY IN THE GUINEA PIG

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THIS study was undertaken at the suggestion of Professor Meyer, primarily for determining the numerical relation between the corpora lutea of pregnancy and implantations in the guinea pig.

Most of the animals used in this experiment were purchased from dealers, for it was impossible, in the short time at my disposal, to obtain young animals of uniform age and with the exception of a few guinea pigs raised in our laboratory, only approximate ages were known.

The guinea pigs were housed in a well-lighted, sunny, heated room. Lantz, '13, reported that the optimum temperature for the guinea pig is 65°. Draper, '20, stated that they thrive best at temperatures between 50° and 70° and found young animals extremely susceptible to small changes in temperature; some of them dying when the temperature was lowered permanently from 60° to 58° F. However, I did not notice any marked difference in the behavior or condition of extremely young animals kept at a temperature of 50°. They showed every sign of vigor and no animals were lost as a result of this exposure. Indeed, I learned of guinea pigs kept in the open in unheated pens, sheltered only from wind and rain. These animals were said to thrive and to multiply at the customary rate, but no records were kept. In my own work I found that a few degrees above or below 50° seemed to make no appreciable difference in the behavior of the animals, and I hence am somewhat sceptical about the marked susceptibility of the guinea pig to cold, so often reported.

The animals were fed dry alfalfa and barley daily and green vegetables about twice a week. Many writers have reported that guinea pigs did not do well on dry feed, but it was my experience that, if fed an abundance of water, they throve on alfalfa and barley alone. Since they are subject to intestinal disturbances, it is of con-

siderable importance that they be fed with the greatest regularity.

Several animals were lost during the course of the experiments and in each case a necropsy was performed. Illness, in several of the animals, extended over a period of weeks. They lost steadily in weight, and tended to assume a characteristically crouching attitude. The fur became rough and tousled. Some of them chewed incessantly, although some pain seemed to be associated with the process. The full significance of this behavior was made clear at the necropsy. Guinea pig No. 7, for example, which succumbed after an illness of three weeks, had an empty stomach, and the abdominal cavity was absolutely devoid of fat. There were no macroscopic. signs of infection or disease. Examination of the teeth revealed that the upper incisors were worn down almost to the gums, with a more than corresponding increase in the length of the lower incisors, making occlusion of the molars impossible. The molars were loose and could easily be picked from the jaw with an ordinary laboratory forceps.

The body of guinea pig No. 12, which died with practically the same symptoms, showed extreme atrophy and emaciation. Ascaroid parasites were found in the rectum. The upper incisors were loose and worn and the short stumps remaining could be be removed with the fingers. The upper and lower incisors were separated by about 8 mm., due to the fact that the molars occluded first and prevented the short, probably fractured incisors from meeting. From the findings in these cases it would seem that guinea pigs may die of starvation because of the presence of worn or irregular teeth and consequent inability to masticate food. It may perhaps be that the changes in the teeth of these animals were due to senility, but further observations are necessary to confirm this before a definite answer can be given to the question.

In order to study daily stages in the pregnancy of guinea pigs it became necessary to mate a large number of animals and to know the exact time of copulation. Stockard and Papanicolaou, '17, studied the œstrous rhythm of

the guinea pig by making microscopic examination of the material found in the vagina. They found that " Guinea pigs kept in a state of domestication and under steady environmental conditions possess a regular diœstrous cycle, repeating itself in non-pregnant females about every sixteen days throughout the entire year, with probably small and insignificant variations during the different seasons. Each period of sexual activity lasts about 24 hours and is characterized by the presence of a definite vaginal fluid which is not sufficiently abundant to be readily detected on the vulva, but is easily observed by an examination of the interior of the vagina." They added that macroscopic signs of heat are unreliable.

In my work it was found impractical to determine the existence of heat microscopically and the knowledge that heat should recur about every fifteen days furnished a starting point. Each female was given a number and entered on an individual record sheet giving the following data:

Date and hour of attempted mating.
Result of attempted mating.
Each time the animal came into heat the record showed:
Whether heat was recognizable by macroscopic examination.
Number of days since last heat.
Number of hours since the first successful coitus.
Number of hours that external signs of heat could be observed by examination.

Matings were attempted daily, whether the animal was supposed to be pregnant or not. The males were introduced into the pens with the females regardless of whether or not the latter were thought to be in heat, and they were allowed to remain with the females from five to fifteen minutes. It was easy to follow the diœstrous cycle of any individual animal. A glance at the guinea pig's record each day showed the number of days since the last heat, and, knowing that heat should return about the fifteenth day, it was practically impossible for it to come and go unnoticed unless it recurred altogether irregularly. We

found that after some practise heat could be determined
rather accurately by inspection. A guinea pig in rut
will often assume the position of copulation when stroked
gently over the lumbar region. The vulva are swollen
and moist, and often a cheesy secretion is seen. The
latter is a positive sign of heat, but we found that some
guinea pigs refused to mate during the entire period in
which the secretion was present.

In young animals we found heat recurring every fifteen
or sixteen days with very little variation among indi-
viduals of the same age. Three striking exceptions in
which heat returned in twelve days will be reported later
in this paper. Papanicolaou and Stockard found that in
old multiparæ the period may be lengthened to 18 days.
I also found that as the animals grow older they seem to
become more and more irregular in their rhythm. In
three very old animals I was unable to find any signs of
heat throughout an entire year, although I attempted to
mate them twice daily. Three other animals maintained
a cycle of 20 days, and in some cases we were unable to
demonstrate any regular œstrous rhythm at all, either by
inspection or by the use of a male.

Subsequently (1920) these workers have reported that
" underfeeding with a diet of 20 grams of carrots per
day produces prolongation of the diœstrum, and at the
same time a congestion in the ovary and uterus and a de-
generation of developing Graafian follicles." They con-
cluded that " the extent of prolongation of the diœstrum
depends upon the stage at which an animal is underfed.
. . . Large follicles seem to require better nutrition than
a small primary follicle. . . . Thus a late underfeeding
has a more injurious effect than an early one, and post-
ponement of the next œstrus is correlated with a postpone-
ment of new ripe follicles in the ovary." Stockard and
Papanicolaou believe that the ovarian follicles are ex-
tremely sensitive to environmental conditions. They be-
lieve that extreme variations in the œstrus cycle of cer-
tain animals may be accounted for, partially at least, by
differences in nutrition.

In the course of these observations the intervals be-

tween attempted matings were shortened, with the idea
that heat might be recurring unnoticed, but mating never
occurred at other intervals. It is doubtful whether any
definite rhythm is maintained by old guinea pigs, for pig
No. 9, which was observed to be in heat December 27,
was not in heat again until 49 days later. Animal No.
20 was in heat October 17 and heat did not return until
91 days later. In another instance heat returned after
118 days. However, since the age of these animals is not
known, it is impossible to be sure that these irregularities
are due to senility.

Bischoff, '44, stated that copulation in the guinea pig
occurs within 3 hours after parturition. In four cases in
which he prevented copulation heat returned after inter-
vals of 40, 50, 51, and 51 days. Hensen, '76, and Rein, '83,
claimed that the most favorable time for copulation is
within one hour after parturition. I observed copulation
in 12 animals immediately after parturition. Matings
were attempted at one-hour intervals for six hours after-
ward. In four cases I was unable to mate the females at
this time. They were found in heat again 34, 36, 81, and
120 days later. The first two animals were about six
months old. The last two were very old, judging by their
teeth. Two females mated 1 hour after parturition, 2
after 2 hours, 1 after 3 hours, 1 after 5 hours, and 2 after
6 hours. In three cases no pregnancy resulted and heat
returned in 31, 31 and 29 days.

Many writers have reported that females refuse the
male shortly after the first copulation. The inference is
that some nervous mechanism automatically terminates
heat soon after copulation. Instances have been reported
in which the female refused the male 20 minutes after the
first copulation. In observations extending over nearly a
year, however, three cases were observed in which the
female mated again eight hours after the first copulation.
In the majority of cases the female permitted copulation
three hours after the first mating. One animal mated 13
times in an interval of 8 hours. It seems that a female
accepts the male at any time during the first stage of heat
regardless of any previous intercourse, but apparently

she permits matings somewhat reluctantly after this. Instead of assuming the position for copulation when approached by the male she often runs around the cage and resists vigorously. Unless the male is very persistent and active copulation will not occur. One female resisted a second coitus for fifteen minutes by kicking, snapping, etc., only to stop suddenly and take the position for copulation. This behavior of the female may be due to previous mating or it may simply mean that the period of heat is subsiding. I am inclined to the latter view, because we have encountered many females among animals which had not been previously mated, who resisted the males vigorously for a time, only to yield in the end. The time during which the females permitted copulation unhesitatingly was a relatively short one, but after this phase had passed the animal might yet be mated if the male was persistent.

Stockard and Papanicolaou, '17, are of the opinion that among domesticated guinea pigs only a slight seasonal variation exists in the occurrence of heat, but in the present series of guinea pigs the fall months were the most favorable for matings, as shown by the following table:

| Month | Number of Matings | Resulting Pregnancies | Percentage |
|---|---|---|---|
| September | 23 | 21 | 91.3% |
| October | 17 | 10 | 58.8 |
| November | 8 | 3 | 37.5 |
| December | 11 | 4 | 36.3 |
| January | 7 | 4 | 57.1 |
| February | 5 | 0 | 0.0 |
| March | 8 | 4 | 50.0 |
| April | 12 | 5 | 41.6 |
| May | 9 | 5 | 55.5 |
| June | 6 | 5 | 83.3 |

The males seemed to be partly responsible for this wide variation. During the winter months they were lethargic and indifferent. When placed in a pen with a female known to be in heat, the male often ignored her, eating unconcernedly instead. In many instances several males had to be placed with such a pig, in succession, before a mating took place. This is in marked contrast to the customary behavior, for when placed in a pen with two

females, the male will often go directly to the female in rut. Sometimes, however, he will mistakenly pursue the one that is not in heat, although repelled by sharp bites and other negations, only to wheel suddenly and mount the receptive female. The pursuit of the wrong animal may only serve to stimulate him, but in some instances it was necessary to remove her before he would turn his attentions to the one in rut. Puzzling sexual idiosyncrasies also were noted. Instances were observed, for example, in which a male would not under any circumstances mate with a certain female which was in heat, although he was persistent in the case of others. On the other hand, some females also were noticed to repulse a certain male, although accepting others.

It will be seen from Table I that 106 matings resulted in 61 pregnancies, or 57.5 per cent. Draper, '20, reported that only 40 per cent. of the animals bred by him became pregnant. Since Stockard and Papanicolaou found 95.4 per cent. out of 88 pigs pregnant, considerable variation would seem to exist. The large discrepancy between their results and ours may be due to the fact that the latter were working with uniformly young, selected animals or that the males were left to remain with the females, instead of being removed after several copulations.

In the many matings not followed by pregnancies, the next œstrous cycle was prolonged. This is shown by the accompanying chart.

| Guinea-pig Number | Heat returned after |
|---|---|
| 21 | 30 days. |
| 32 | 44 |
| 22 | 28 |
| 27 | 15 |
| 50 | 46 |
| 52 | 29 |
| 8 | 30 |
| 9 | 31 |
| 39 | 12 |
| 37 | 15 |
| 3 | 29 |
| 5 | 30 |
| 8 | 15 |
| 16 | 30 |

As noticeable in the above chart, the lengthened diœstrous periods are nearly exact multiples of 15, the normal period, thus showing that the cycle is definitely periodic as reported by Stockard and Papanicolaou, '17*b*. Long, '15, found that the œstrous cycle was prolonged by inserting a glass rod in the vagina of the rat. He held this prolongation to be due to a stimulation of the cervix of the uterus. Although I stimulated the uterus of guinea pigs by means of a warm glass rod in three cases only, heat returned in 15, 15 and 16 days, and I regret that I was not able to extend this series of experiments in order to obtain more data on this interesting phenomenon revealed by Long in the rat. However, from the above table, it is clear that copulation definitely prolongs the next œstrous cycle in the majority of cases. This may be due to direct stimulation of the cervix of the uterus, as explained by Long, or implantation may have occurred, followed by abortion or by absorption of the young conceptuses, in cases in which the period was greatly prolonged.

Guinea pig No. 39 (see Table II) was mated two hours after parturition, but no pregnancy followed. This animal was remated 12 days later, with resulting pregnancy. This confirms a case reported by Rubasckhin, '05, in which heat returned 10 days after parturition. Stockard and Papanicolaou, in considering Rubasckhin's report, regarded 10 or 12 days as too short a period to indicate the return of heat. Nevertheless, in the case reported here heat was unmistakable, and this animal which was mated 12 days after parturition became pregnant. I observed heat to return in 12 days also in two other pigs.

Young animals constantly in association with males became pregnant at an earlier age than females isolated from males. Of a litter containing 3 females and 1 male, two females were placed in separate cages a few days after birth and the remaining male and female were allowed to run together. At the age of 5 months, the latter produced a litter. This indicates that the mating of this pair occurred before the animals were three months old. Yet no ill effects of this early mating or of the inbreeding could be detected in the offspring.

When the two sisters were two months old, males were introduced into the pens twice daily, but no signs of heat were observed, and no matings occurred until these females were five months old. Similar results were obtained with two other litters. Since my work was done Mr. Warnock, a fellow student, has observed two females to bear viable litters at the end of the third month. This implies mating at the early age of one month. The paternal male was several months older, however.

### The Corpora Lutea of Pregnancy

In order to study the correlation between corpora lutea and implantations during the various stages of pregnancy, animals were mated and killed, from the seventh day of gestation on, for each day up to and including the fifteenth. From the fifteenth day to full term, animals were killed every other day.

When the guinea pigs were killed, the ovaries and uteri were removed and placed in formalin for twenty-four hours and the number of embryos in each horn of the uterus recorded. The ovary corresponding to the horn of the uterus having the larger number of conceptuses was arbitrarily chosen for use in determining what relation might exist between the number of conceptuses and the number of corpora lutea. Thus guinea pig No. 10 had two conceptuses in the right horn and one in the left. The right ovary was embedded and cut serially into thick sections. The left ovary was cut 7 micra thick for the study of changes in the corpora lutea during pregnancy.

In a study of 14 embryos, Draper, '20, found 76 in the left horn and 69 in the right, a ratio of 1 to 0.9. Of 98 embryos from 35 guinea pigs, I found 55 in the right horn and 43 in the left, a ratio of 1 to 0.78. The average number of fœtuses per pregnancy was three.

Table II shows that there is a marked agreement between the number of corpora lutea in an ovary and the number of implantations in the corresponding horn of the uterus. Out of 34 ovaries examined, the number of corpora lutea was the same as the number of embryos

in the corresponding horn of the uterus in all save six cases. In five of these six instances there was one embryo less in the horn of the uterus than there were corpora lutea in the ovary. In the other case, the right

TABLE II

| Guinea Pig | Duration of Pregnancy | Embryos | | Corpora Lutea | | Remarks |
|---|---|---|---|---|---|---|
| | | Right | Left | Right | Left | |
| 35......... | 7 | 1 | 1 | 3 | 1 | Well-formed C.L. but |
| 34......... | 8 | 0 | 3 | 1 | 3 | no external evidence |
| 33......... | 9 | 2 | 1 | 2 | 1 | of implantations. |
| 32......... | 10 | 1 | 3 | 1 | 3 | |
| 31......... | 11 | 1 | 1 | 1 | 3 | Well-marked evidence |
| 30......... | 12 | 3 | 0 | 3 | 0 | of resorption. |
| 29......... | 13 | 1 | 2 | 1 | 2 | |
| 28......... | 14 | 3 | 1 | 3 | 1 | |
| 27......... | 15 | 0 | 1 | 0 | 2 | |
| 26......... | 17 | 3 | 0 | 2 | 0 | |
| 25......... | 19 | 1 | 3 | 1 | 3 | |
| 24......... | 21 | 2 | 1 | 2 | 1 | |
| 23......... | 23 | 2 | 3 | 2 | 3 | |
| 22......... | 25 | 3 | 0 | 3 | 0 | |
| 21a........ | 27 | 3 | 0 | 2 | 0 | |
| 20......... | 29 | 3 | 1 | 3 | 1 | |
| 19......... | 31 | 2 | 1 | 2 | 1 | |
| 18......... | 33 | 2 | 0 | 3 | 1 | |
| 17......... | 35 | 2 | 1 | 2 | 0 | |
| 16......... | 37 | 3 | 1 | 3 | 1 | |
| 15......... | 39 | 2 | 1 | 2 | | |
| 14......... | 41 | 1 | 2 | 1 | 2 | |
| 13......... | 43 | 1 | 2 | 1 | 2 | |
| 12......... | 45 | 1 | 2 | 2 | 2 | Conceptus on left side |
| 11......... | 47 | 1 | 1 | 2 | 1 | almost completely re- |
| 10......... | 49 | 2 | 1 | 2 | 1 | sorbed. |
| 9.......... | 51 | 2 | 1 | 2 | 1 | |
| 8.......... | 53 | 1 | 2 | 1 | 2 | |
| 7.......... | 55 | 2 | 0 | 2 | 0 | |
| 6.......... | 57 | 1 | 1 | 2 | 1 | |
| 5.......... | 59 | 1 | 2 | 2 | 2 | |
| 4.......... | 61 | 2 | 1 | 2 | | |
| 3.......... | 63 | 3 | 1 | 3 | | |
| 2.......... | Term | 2 | 1 | 2 | 1 | |
| { A........ | 23 | 0 | 0 | 2 | 0 } A | C.L. of pregnancy but |
| { B........ | 45 | 0 | 0 | 1 | 1 } B | no implantations found. |

horn showed 3 embryos although only two corpora lutea of pregnancy were present in the ovary. Hence, in this case, two embryos developed from a single ovum or a single follicle contained two ova. In the instances where there was one more corpus luteum than embryos it is possible that another conceptus was present and became

absorbed or that an ovum degenerated before implantation, or that it failed of fertilization.

As shown by Meyer, '17 and '19, and Stockard and Papanicolaou, '18, absorption is not uncommon in the uteri in guinea pigs. In this series, three embryos which were clear-cut cases of absorption were found upon examination of the uteri after their removal. In No. 12, which was killed forty-five days after copulation, two normal embryos were found in the left horn, but in the right horn there was nothing but a small mass which had undergone almost complete absorption.

According to Stockard and Papanicolaou, '18, embryos eight or ten days old may be detected by '' carefully feeling the uterus through the body wall of the mother.'' They report a case as follows:

A normally developed embryo 19 mm. crown rump length is shown in Fig. 6 and near it is seen an amorphous embryonic mass 2 mm. in longest diameter which represents the other member of the litter. . . . The entire mass of the smaller ovum in the uterus was about that of a ten-day specimen, while the normal individual was a typical 20-day specimen. This case was detected by external examination and was merely opened in order to use the embryos for illustrating the phenomenon.

Although I used the method of Papanicolaou and Stockard in palpating guinea pigs, in no instance was I able to determine the number of embryos with certainty under fifteen days. Because of this fact, I found it necessary to sacrifice the animals in order to determine the number of implantations before this period.

Guinea pig No. 35 and guinea pig No. 34 were killed seven and eight days after conception, respectively, and the uteri removed. Careful palpation of the removed uteri failed to reveal the number of conceptuses. The uteri were then opened, but in order to determine the number of implantations present it was necessary to embed them and make serial sections. From this I am led to question the possibility of determining the number of embryos in the uterus by palpation through the abdominal wall on the eighth to tenth day of pregnancy. This skepticism seems warranted, further, by the measurements of

three ten-day conceptuses, *6.5 × 3 mm.*; *6.8 × 4.5 mm.*; *6.5 × 4.5 mm.* respectively. Draper gave the estimated length of an 11-day *embryo* measured under magnification as 2 mm.

Stockard and Papanicolaou (1918) likewise reported that a "slightly cystic ovary" has frequently been diagnosed by palpation through the abdominal wall of the guinea pig. In my observations 23 out of 75 ovaries were found to be cystic; but the largest cyst measured only *1.6 mm.× 1.68 mm.* and not even this could by any chance have been palpated through the abdominal wall. Hence, it would seem that Stockard and Papanicolaou must have been dealing with markedly large and unusual, rather than with slightly, cystic ovaries.

From a study of a large series of gestations in the domestic pig, Corner, '21, concluded that internal migration of ova is relatively common. This small series of pregnancies in the guinea pig furnishes very little evidence upon this question, for such a possibility is suggested only by No. 17, a pregnancy of 35 days in which there were 2 corpora and 2 implantations on the right side and no corpora but one implantation on the left side. Since the total number of implantations in this case exceeds that of corpora, one must assume that one ovum divided or that one follicle contained ova and that one of the ova arising from the right then migrated to the left cornu. However, since this pregnancy was so far advanced, this assumption implies that a corpus luteum of pregnancy in the guinea pig can not be wholly resorbed in 35 days and that it never fails to form.

It is of special interest in this connection that a second case of this kind has been observed in this laboratory by Miss Clark. In this case there were two corpora in the left ovary and none in the right, with one implantation on each side. Since this pregnancy was only 17 days old, the question of early resorption of the corpus luteum probably can be excluded with considerable certainty but that of failure of the corpus luteum to form, remains.

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# VARIATIONS IN THE NUMBER OF VERTEBRÆ AND OTHER MERISTIC CHARACTERS OF FISHES CORRELATED WITH THE TEMPERATURE OF WATER DURING DEVELOPMENT

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## I

FOR several years I have been studying the correlations between altered environmental conditions and the number of vertebræ and other segmentally arranged structures in fishes. Johannes Schmidt, of the Carlsberg Laboratory in Copenhagen, has been carrying on a series of intensive investigations (see bibliography) which deal with the same problem, and which are for the greater part rather closely paralleled by my own studies. Both of us have obtained, independently, a rather large volume of experimental and observational evidence indicating that the meristic characters displayed by an individual fish are determined not alone by heredity, but in part also by the environmental conditions, particularly temperature, which prevail during some sensitive developmental period.

## II

The present study is one of those comprising the series just mentioned. It deals with variations in the number of vertebræ, scale-rows and fin-rays within one year-class and between two successive year-classes of the lake " shiner," *Notropis atherinoides* (Cyprinidæ), and in comparison between the corresponding year-classes of the " blue-gill " sunfish, *Lepomis incisor* (Centrarchidæ). These variations appear to be correlated with differences in temperature prevailing during the several developmental periods involved.

The material of each species is probably a unit as re-

gards "race." It was all obtained in a lagoon in Jackson Park, Chicago, during the third week of December, 1919. At this time what seemed to be the entire fish population of the lagoon was congregated in an opening, about five meters wide, in the ice along shore. These fishes showed symptoms of asphyxiation. They were so abundant that at times, while they were gyrating about, the mass of fishes below would force the almost solid upper layer a centimeter or two above the surface over an area of perhaps a square meter. A water bucket was filled with fishes, mostly *Notropis atherinoides,* by two or three sweeps of a small hand-net. More than one thou-
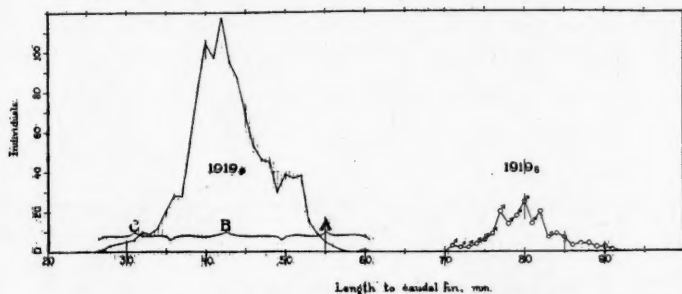


FIG. 1. Frequency graph, indicating the year-classes of *Notropis atherinoides.*

sand of the young of that year (1919) of the Notropis were saved after random selection, and preserved for study with all older fish of the same species. All of the sunfishes (*Lepomis incisor*) obtained at the same time and place were preserved and studied. Of the two species, the sunfishes belonged to a population practically confined to the lagoon, while the minnows had moved into the lagoon, late in the preceding autumn, from the more open waters of Lake Michigan.

The specimens thus obtained were grouped into year-classes. Age determinations were made by the usual methods of counting the annuli (winter lines) on the scales, and as a check the seasonal bands of the otoliths, and furthermore by the preparation of a frequency graph

from the length measurements of the entire material.
The young of the year (obtained in 1919) are referred to
as the $1919_9$ class; those of the previous year as the $1919_8$
class, and so forth. The $1919_9$ year-class of the *Notropis
atherinoides* is further divided into three subclasses, A,
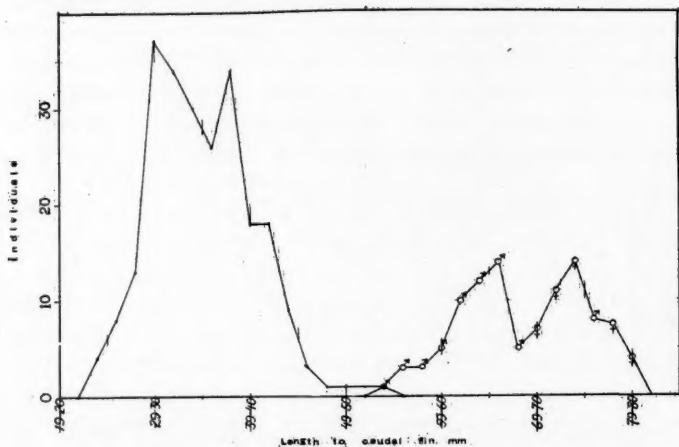B and C, named in the direct order of hatching, hence in



FIG. 2. Frequency graph illustrating the year-classes of *Lepomis incisor*.

the indirect order of size. The year-classes for both spe-
cies are indicated on the graphs forming Figs. 1 and 2.
The symbols on the curve for the $1919_8$ class of each spe-
cies indicate the sex predominant among the representa-
tives of each size.

## III

A series of water temperatures appear unavailable, but
in the case of such a shallow, nearly enclosed lagoon the
air temperatures of the region may safely be substituted.
Hence the *Climatological Data* (Illinois Division, 1918
and 1919) for Chicago were used in constructing Fig. 3;
the temperatures given for each week were obtained by
averaging the daily means.

On the temperature chart there are indicated the
periods of development for each of the two species as ob-

served at the same locality in 1919. The data for *Lepomis incisor* seem satisfactory (see Hubbs, 1919), but those for *Notropis atherinoides* are less complete and more circumstantial. In the case of the minnow, the developmental period is divided into three periods (A, B and C) corresponding with the three subclasses into which the 1919 year-class has been divided. Period A followed an inshore spawning migration of the mature individuals, coincident with the rapid rise in temperature during March; period C preceded the withdrawal of the breeding stock from the shore waters of the lake; the intervening period is termed B.

The limited field observations on the spawning and developmental period for *Notropis atherinoides* during 1919 are, fortunately, strongly confirmed by a study of
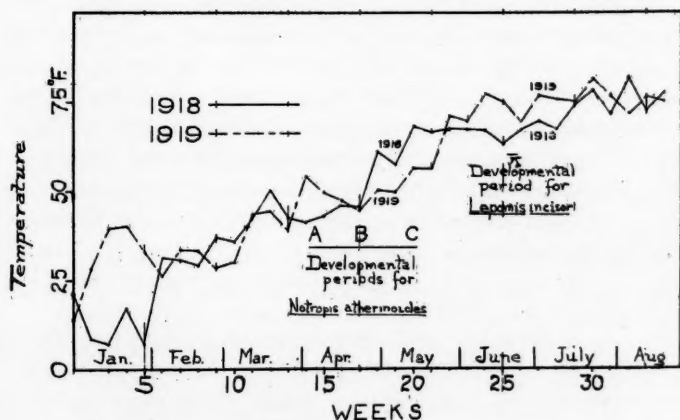


FIG. 3.   Air temperature at Chicago, 1918–1919.

the scales. The scales of the largest specimens taken in December, namely those comprising subclass $1919_9A$, and forming a distinct mode in the frequency graph (Fig. 1), show a well marked nuclear area of weak concentrated circuli indicative of retarded growth, followed by the coarser, more regular circuli indicative of normal summer growth. This initial period of retarded growth presum-

ably corresponds with the cold period in April (see Fig. 3). The scales of the medium-sized specimens (subclass B) show on the average a narrower nuclear area suggesting slackened growth. It is presumed that these individuals passed through their early development toward the end of this cold period. The scales of the smallest specimens, those of subclass C, show no such nuclear area of weak concentrated circuli. These fishes supposedly developed during the warm weather of May.

The data on the developmental period of these two species for the preceding breeding season (1918) are less complete than those for 1919, yet not wholly lacking. *Lepomis incisor,* at least, bred during the corresponding weeks in both years (but in less abundance in 1918 than in 1919).

A comparison of the available observational data with the temperature chart (Fig. 3) indicates that, on the average, the developmental period for *Notropis atherinoides* was colder in 1919 than in 1918, whereas these temperature relations were distinctly reversed in the case of *Lepomis incisor,* and furthermore, that the temperature was distinctly higher at the beginning and toward the close of the 1919 breeding season for the Notropis, than during the middle of this period.

## IV

These differences in the developmental temperature appear to be correlated with variations in the number of segments in the case of both fishes. Comparisons will first be made between the two year groups of *Notropis atherinoides,* then between the same year groups of *Lepomis incisor,* and finally between the three subclasses into which the 1919 brood of the Notropis has been divided.

The vertebræ in the $1919_9$ class of *Notropis atherinoides* are sufficiently more numerous on the average than those of the $1919_8$ class to shift the modal number from 41 to 42, the average from 41.41 ($\pm 0.04$)[1] to 41.74 ($\pm 0.015$).

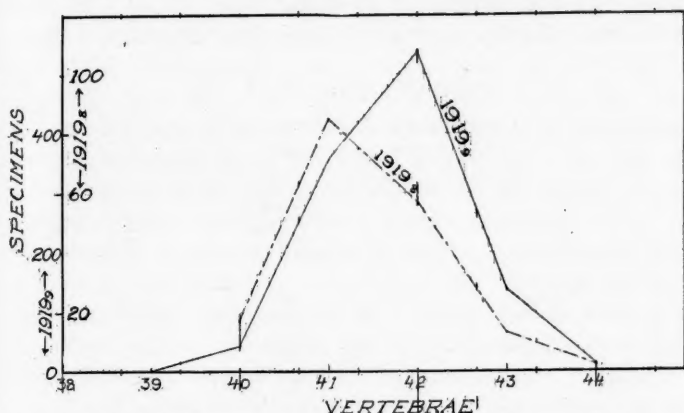[1] The probable error of the average.

Fig. 4.  Comparison of number of vertebræ in successive year-classes of *Notropis atherinoides*.

The portion of the vertebral column affected is the caudal, not the precaudal (abdominal) division: the averages for the precaudal vertebræ are 22.82 ($\pm$ 0.02) for $1919_8$ and 22.85 ($\pm$ 0.01) for $1919_9$ for the caudal vertebræ, 18.60 ($\pm$ 0.035) for $1919_8$, and 18.87 ($\pm$ 0.01) for $1919_9$.  Similarly, the number of scales in the lateral line averages higher in the $1919_9$ lot: the modal number is 40 rather than 39 as it is in $1919_8$ class; the average number is 40.05 ($\pm$ 0.04) rather than 39.65 ($\pm$ 0.04).  The modal number
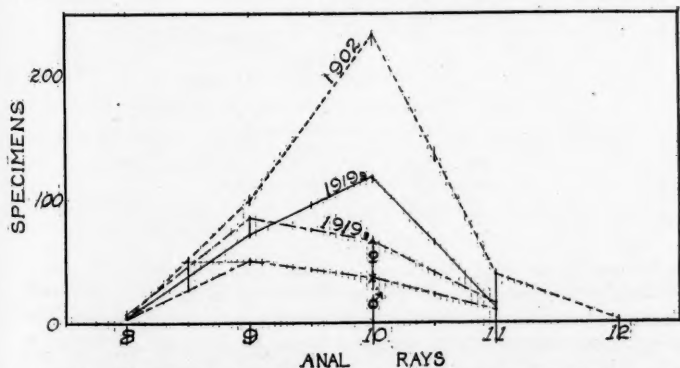


Fig. 5.  Comparison of variations in number of branched and anal rays in different year-classes of *Notropis atherinoides*.

of branched anal rays[2] is 10 in the $1919_9$ series, 9 in the $1919_8$ class; the averages are 9.52 ($\pm$ 0.05) for $1919_8$ males,

<div align="center">FREQUENCY TABLE I</div>

<div align="center">Comparison of the Meristic Features of the 1918 and 1919 Broods of *Notropis atherinoides*</div>

| Year-class | Character | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total Number of Vertebræ | | | | | | | | | |
| | 39 | 40 | 41 | 42 | 43 | 44 | | | | |
| $1919_8$ | — | 17 | 85 | 57 | 13 | 2 | | | | |
| $1919_9$ | 1 | 43 | 356 | 539 | 137 | 12 | | | | |

| Year-class | Number of Precaudal Vertebræ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 21 | 22 | 23 | 24 | 25 | | | | |
| $1919_8$ | 3 | 38 | 121 | 13 | — | | | | |
| $1919_9$ | 2 | 240 | 766 | 78 | 1 | | | | |

| Year-class | Number of Caudal Vertebræ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 17 | 18 | 19 | 20 | 21 | | | | |
| $1919_8$ | 6 | 74 | 80 | 16 | — | | | | |
| $1919_9$ | 6 | 269 | 661 | 142 | 10 | | | | |

| Year-class | Number of Scales in Lateral Line | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 |
| $1919_8$ | 2 | 26 | 95 | 92 | 34 | 11 | 2 | — | — |
| $1919_9$ | — | 22 | 90 | 164 | 66 | 21 | 9 | 2 | 1 |

| Year-class | Number of Branched Anal Rays | | | | |
|---|---|---|---|---|---|
| | 8 | 9 | 10 | 11 | 12 |
| 1902 | 4 | 100 | 232 | 39 | 2 |
| $1919_8 \male$ | 4 | 51 | 37 | 10 | — |
| $1919_8 \female$ | 3 | 34 | 29 | 6 | — |
| $1919_9$ | 4 | 72 | 118 | 12 | 1 |

[2] The last ray as usual was counted as double, *i.e.*, as divided to the base. Occasionally the posterior half of this divided ray is again divided well toward the base. In fact a complete transition can be traced between fins having a given number of rays with those having one more ray. It is highly improbable, however, that this transition is sufficiently frequent as to permit a serious modification of the average number of rays, through a personal error in counting.

9.53 ($\pm$ 0.06) for 1919$_8$ females, and 9.69 ($\pm$ 0.03) for both
sexes of the 1919$_9$ class; in material collected in 1902 in
the same lagoon the average is still higher, 9.83 ($\pm$ 0.02).
The data on which these figures are based is given in Fre-
quency Table I. In all three characters, namely the num-
ber of vertebræ, of scales along the lateral line, and of
branched anal rays, the year-class developed in the cooler
season displays a significantly higher average.

A highly similar yet exactly reverse condition is dis-
played in the analysis of the counts on the *Lepomis in-
cisor* material. In this case the total number of vertebræ,
and the number of caudal, but not precaudal, vertebræ;
the number of dorsal spines, dorsal soft-rays, anal soft-
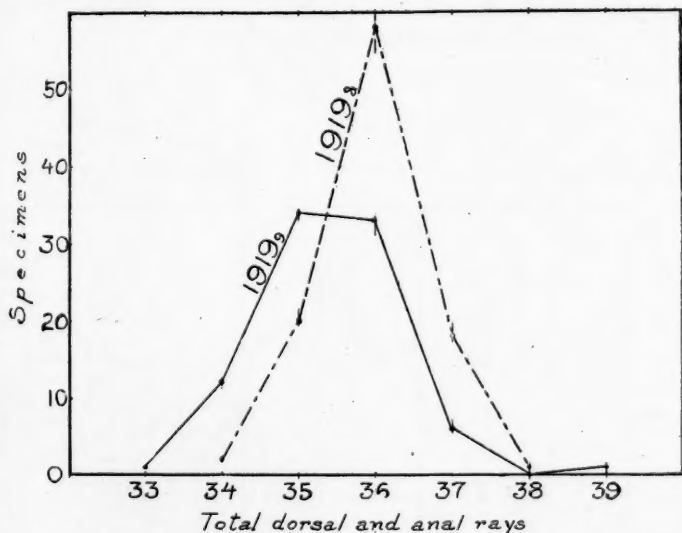rays, and hence the total number of vertical fin-rays, all

FIG. 6. Comparison of number of dorsal and anal fin rays in successive year-
classes of *Lepomis incisor*.

average higher in the class born in 1918 than in that of
1919. But we noted above that the temperature relations
during the developmental periods of the two years were
likewise reversed. In both *Notropis atherinoides* and

## FREQUENCY TABLE II

COMPARISON OF THE NUMBER OF VERTEBRÆ IN THE 1918 AND 1919 BROODS OF
*Lepomis incisor*

| Year-class | Character | | | Average | Probable Error |
|---|---|---|---|---|---|
| | Total Number of Vertebræ | | | | |
| | 28 | 29 | 30 | | |
| 1919₈............ | — | 95 | 9 | 29.10 | 0.02 |
| 1919₉............. | 7 | 219 | 8 | 29.00 | 0.00 |
| | Number of Precaudal Vertebræ | | | | |
| | 11 | 12 | 13 | | |
| 1919₈............ | 2 | 100 | 2 | 12.00 | 0.01 |
| 1919₉............ | 2 | 230 | 2 | 12.00 | 0.00 |
| | Number of Caudal Vertebræ | | | | |
| | 16 | 17 | 18 | | |
| 1919₈............ | — | 95 | 9 | 17.09 | 0.02 |
| 1919₉............ | 7 | 219 | 8 | 17.00 | 0.01 |
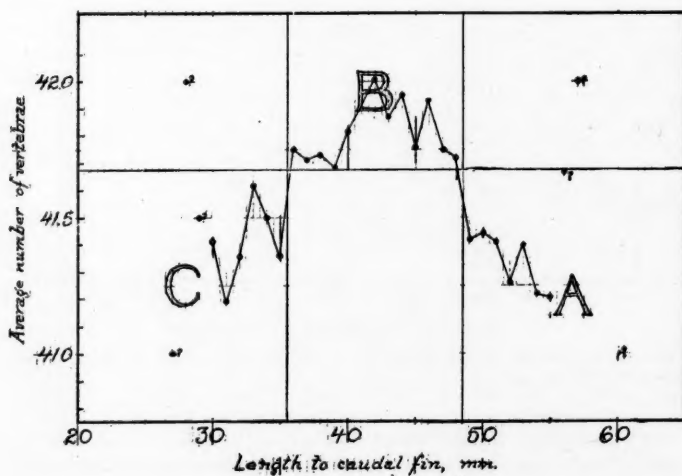


FIG. 7. Illustrating seasonal variation in number of vertebræ in *Notropis atherinoides*.

*Lepomis incisor,* therefore, a higher number of segments was developed in the year class developed at the lower temperature. The detailed data are given in Frequency Tables II and III.

Evidence has already been given indicating that the 1919 year-class of *Notropis atherinoides* is divisible into three subclasses, of which the middle (B) developed during colder weather than either the first (A) or the last (C). The data given in Frequency Table IV and in figure 6 demonstrate that this subclass B possesses a decidedly higher number of vertebræ than either of the other two. The averages are as follows: for the 146 specimens of subclass A, 41.38 ($\pm$ 0.04); for the 845 comprising subclass B, 41.84 ($\pm$ 0.02); for the 97 individuals of subclass C, 41.42 ($\pm$ 0.05).

### FREQUENCY TABLE III

COMPARISON OF THE NUMBER OF FIN-RAYS IN THE 1918 AND 1919 BROODS OF *Lepomis incisor*

| Year-class | Character | | | | Average | Probable Error |
|---|---|---|---|---|---|---|
| | Number of Dorsal Spines | | | | | |
| | IX | X | XI | XII | | |
| 1919₈....... | 1 | 79 | 22 | — | 10.21 | 0.03 |
| 1919₉....... | 2 | 74 | 11 | 1 | 10.125 | 0.03 |
| | Number of Dorsal Soft-rays | | | | | |
| | 10 | 11 | 12 | 13 | | |
| 1919₈....... | 1 | 37 | 63 | — | 11.61 | 0.03 |
| 1919₉....... | 3 | 51 | 32 | 2 | 11.375 | 0.04 |
| | Number of Anal Soft-rays | | | | | |
| | 9 | 10 | 11 | 12 | | |
| 1919₈....... | — | 2 | 80 | 19 | 11.17 | 0.03 |
| 1919₉....... | 1 | 12 | 70 | 5 | 10.90 | 0.03 |

| Year-class | Total Rays in Dorsal and Anal Fins | | | | | | | Average | Probable Error |
|---|---|---|---|---|---|---|---|---|---|
| | 33 | 34 | 35 | 36 | 37 | 38 | 39 | | |
| 1919₈....... | — | 2 | 20 | 58 | 18 | 1 | — | 35.96 | 0.05 |
| 1919₉....... | 1 | 12 | 34 | 33 | 6 | — | 1 | 35.40 | 0.07 |

## FREQUENCY TABLE IV

Variation in Number of Vertebræ within One-year Class of
*Notropis atherinoides*

| Sub-class | Size Group | Number of Vertebræ | | | | | | Average | Probable Error |
|---|---|---|---|---|---|---|---|---|---|
| | | 39 | 40 | 41 | 42 | 43 | 44 | | |
| $1919_2$ C.... | 27 | — | — | 1 | — | — | — | (41.00) | — |
| | 28 | — | — | 1 | 1 | 1 | — | (42.00) | 0.32 |
| | 29 | — | — | 2 | 2 | — | — | (41.50) | 0.29 |
| | 30 | — | — | 9 | 4 | — | — | 41.31 | 0.09 |
| | 31 | — | 1 | 5 | 3 | — | — | 41.22 | 0.16 |
| | 32 | — | 2 | 7 | 4 | — | 1 | 41.36 | 0.175 |
| | 33 | — | 1 | 4 | 7 | 1 | — | 41.62 | 0.145 |
| | 34 | — | 1 | 7 | 10 | — | — | 41.50 | 0.095 |
| | 35 | — | 1 | 13 | 7 | 1 | — | 41.36 | 0.09 |
| $1919_2$ B.... | 36 | — | — | 11 | 13 | 4 | — | 41.75 | 0.09 |
| | 37 | — | 1 | 16 | 18 | 6 | — | 41.71 | 0.07 |
| | 38 | — | 5 | 10 | 36 | 5 | — | 41.73 | 0.05 |
| | 39 | 1 | 4 | 24 | 39 | 8 | 1 | 41.68 | 0.06 |
| | 40 | — | 3 | 28 | 44 | 17 | — | 41.82 | 0.05 |
| | 41 | — | 2 | 24 | 46 | 16 | 2 | 41.91 | 0.055 |
| | 42 | — | 1 | 21 | 67 | 18 | 3 | 42.01 | 0.045 |
| | 43 | — | 3 | 24 | 41 | 17 | 1 | 41.87 | 0.06 |
| | 44 | — | 1 | 18 | 43 | 14 | 1 | 41.95 | 0.05 |
| | 45 | — | 2 | 21 | 31 | 6 | 2 | 41.76 | 0.07 |
| | 46 | — | — | 13 | 23 | 10 | — | 41.93 | 0.07 |
| | 47 | — | — | 14 | 23 | 2 | 1 | 41.75 | 0.06 |
| | 48 | — | 1 | 16 | 16 | 7 | — | 41.72 | 0.08 |
| $1919_2$ A.... | 49 | — | 4 | 6 | 14 | — | — | 41.42 | 0.10 |
| | 50 | — | 3 | 14 | 16 | 1 | — | 41.44 | 0.10 |
| | 51 | — | 2 | 14 | 9 | 2 | — | 41.41 | 0.095 |
| | 52 | — | 2 | 16 | 9 | — | — | 41.26 | 0.075 |
| | 53 | — | 2 | 6 | 6 | 1 | — | 41.40 | 0.14 |
| | 54 | — | — | 7 | 2 | — | — | 41.22 | 0.09 |
| | 55 | — | 1 | 2 | 2 | — | — | 41.20 | 0.225 |
| | 56 | — | — | 1 | 2 | — | — | (41.67) | 0.18 |
| | 57 | — | — | — | 1 | — | — | (42.00) | — |
| | 58 | — | — | — | — | — | — | — | — |
| | 59 | — | — | — | — | — | — | — | — |
| | 60 | — | — | 1 | — | — | — | (41.00) | — |

## V

It has generally been taken for granted, as a basic assumption, that such differences as those here shown to hold between two successive year-classes, and between successive groups within a single year-class, are indicative of racial distinction. Obviously this assumption can not be maintained as wholly true. Moenkhaus (1895, 1898) indeed long ago demonstrated the occurrence of a significant annual variation within one race of fishes (in the case of the darters *Percina caprodes* and *Boleosoma*

*nigrum*).  Schmidt (1921) has lately studied such annual
fluctuations in great detail in *Zoarces,* and has induced
like changes by experimental control of temperature in
*Lebistes* (1919*a*, 1919*b*) and *Salmo* (1921).  I have ob-
tained similar experimental results for coregonine fishes
and for *Esox lucius* (data yet unpublished).

On the other hand it has been clearly demonstrated in
a number of cases that fine "racial" differences are in-
herited.  Thus Schmidt (1917*a*, 1917*b*, 1918, 1920, 1921)
has determined by his "offspring analyses" that a high
degree of positive correlation holds between the number
of segments and other features of the maternal parent
and the unborn embryos of *Zoarces.*  Similar results
were obtained by Punnett (1904) for the viviparous
shark, *Etmopterus [Spinax] niger.*  In *Salmo,* Schmidt
(1919*c*) has lately demonstrated that the finer differences
in the number of vertebræ of both parents are inherited,
and in the viviparous teleost *Lebistes reticulatus,* the
same author has found (1919*a*, 1919*b*) that minor varia-
tions in the parental number of dorsal fin-rays are in-
herited.  In somewhat similar fashion Sumner (1918,
etc.) has demonstrated that subspecific differences in
color and size in the mouse genus *Peromyscus* are in-
herited, even under changed enviromental conditions.  A
considerable body of indirect observational evidence
might be brought forward, if needed, in confirmation of
the assumption that these fine racial differences are in-
herited.

Clearly the same sort of variations as are induced by
altered environmental conditions do characterize geneti-
cally distinct local races of fishes.  Furthermore, these
two sets of correlations display certain striking simi-
larities or analogies, the significance of which the writer
is attempting to determine in the series of studies of
which the one here reported is a part.

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# STUDIES ON FISH MIGRATION II. THE INFLUENCE OF SALINITY ON THE DISPERSAL OF FISHES[1]

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IN connection with an extensive study of the factors influencing fish migration, certain experiments were performed during the summers of 1919 and 1920 to determine the effects of different salinities on the reactions of fish under laboratory conditions. Besides testing the animals with the salts of sea water, preliminary experiments were made with changed temperature and stream flow.

## MATERIAL AND METHODS

The apparatus consisted of a two-tributary unit of a river system so arranged that different solutions could be introduced, affording the fish an opportunity to select the more favorable one. Two almost parallel troughs were so directed as to let the solutions flow down into a long receiving trough that had adjustable outlets in the middle.

There was also an intake at the extreme end of the large receiving trough so that if desired three intakes could be used. When only the two converging troughs were supplied with currents, a partition was placed across the middle of the receiving trough so that the water could flow laterally and eventually escape from the pool by the regular outlet.

The two tributary troughs were each 10 feet long, 4 inches deep and 4½ inches wide and the receiving trough was 10 feet long, 8 inches deep and 8¾ inches wide. The twin troughs were marked off in feet and conspicuously

[1] Contribution from the Biological Laboratory of the U. S. Bureau of Fisheries at Woods Hole, Mass.

labeled at the proper points so that from a single ob-
servation post, record could be taken of the distances
traveled by fishes responding to the streams flowing down
the incline.

Streams were introduced after temporary storage in
two barrels located above the ends of the experimental
troughs. In some experiments the inflowing currents
came directly from the circulation pipes of the laboratory.

Experiments were performed with sea water, fresh
water and combinations of the two, followed by tests with
the individual salts of sea water in $m/10$ solutions. Tem-
perature and stream flow were varied and proved most
important adjuncts to the salts in affecting behavior.

In order to be quite certain that habit formation as a
factor was eliminated, it was customary to select a trough
used during the night for sea water inflow and introduce
a substance less attractive, for the first few experiments
with a group. As conditions of illumination were uni-
form and the troughs were so near each other, this proce-
dure probably reduced the error due to a habit factor.

The fish were males, selected for apparent vigor and
averaged about 12 centimeters in length. They were used
for a complete series of experiments in lots of ten, then
replaced by another ten of similar size. In the majority
of the experiments, the species used was *Fundulus hete-
roclitus*. Its habits throughout the year were already
known to the writer (1916, 1920). Loeb, Thomas and
others had already studied its susceptibility to toxic sub-
stances. It is anadromous, highly resistant, yet furnishes
quick reactions.

*Fundulus majalis* was used less frequently as it is not
so resistant to laboratory conditions and behaves differ-
ently with reference to tides. The observations of Mast
(1915) made it especially desirable to study the reactions
to currents and accordingly a series of experiments was
made.

*Clupea harengus* dies quickly in captivity. Its re-
sponses are extremely delicate and it has been used quite

successfully by Shelford, Powers and others in experiments on temperature, acidity, alkalinity and salinity. It proved too excitable for the experiments with which the present work was concerned.

### EXPERIMENTS

*Fresh Water and Sea Water.  (Temperature 20° C.)*

With apertures $\frac{3}{8}$ in. in diameter in two glass tubes directing horizontal streams of fresh water and sea water to a point six inches from the ends of the experimental troughs, it was found that 10 fish responded during 25 trials in such a manner that 11.8 was the value for responses to fresh water and 44.6 was the value for the sea water.  These figures were obtained by multiplying the number of fish responding by the feet traveled up the trough towards the current, adding the total of 25 trials and securing averages for control and experiment.

The fish responded readily to the flow of water and since there was an admixture of fresh and salt water in the lower ends of the troughs, they did not at first discriminate the sea water before reaching a point 6 or 7 feet from the pool, that is 3 or 4 feet from the intake. As their reactions to the currents became established, however, they came in smaller numbers and finally became aligned along the sea water current at a distance of not more than a foot from the intake.

On changing the flow of fresh water to salt and vice versa, it was noted that at first the fish came into the trough formerly salt, and proceeded beyond the point where they usually traveled in fresh water.  This was in part due to the habitual response and partly to the presence of some salts in the trough.  On reaching the intake, they rapidly returned to the pool, one or two pioneered in each trough, then the whole group explored the salt trough and finally came to a point near the salt water intake.

## Reactions to Salts in Solution

A preliminary series of experiments was run with fish immersed in $m/10$ solutions of the salts of sea water, made up in fresh water. Results were obtained similar to those recorded by Loeb, Thomas and others with fish and corresponding ones known to the writer from experiments with the larvæ of mosquitoes (1916).

By using the barrels above the experimental troughs solutions of the salts individually and in combination were introduced into the apparatus, with fresh water or sea water run as the control current. At first temperature and stream pressure were kept constant. The temperature averaged 20.5° C. and the pressure was sufficient to send the currents horizontally to a distance of six inches from the ⅜-in. glass tubes.

The reactions to individual salts as compared with fresh water are shown in the table below, only the averages at the end of 25 trials with 10 fish being recorded.

RESPONSES OF FISH TO SALTS

| | | |
|---|---|---|
| $MgSO_4$ | 46 | Control, 0 |
| NaCl | 22.6 | Control, 1 |
| $CaCl_2$ | 6 | Control, 20 |
| $MgCl_2$ | 5.7 | Control, 21.5 |
| KCl | 2 | Control, 15 |

It is quite evident that with temperature and stream pressure the same, *Fundulus heteroclitus* will react quite definitely to salts. It is attracted to the less toxic ones, $MgSO_4$, and NaCl, and is repelled by those that are most toxic to it.

Similar experiments with sea-water solutions and sea water as the control current brought out quite clearly that for the species used, $m/10$ solutions of the more toxic individual salts were not strong enough to repel the fish. For example in the case of the most toxic, KCl, the score for 25 trials with 10 fish was 43 for the control sea water and 34 for the experimental current with KCl in $m/10$ solution.

Likewise, combinations of the salts showed only too

well the attractiveness of the mixed solutions. With an
$m/10$ solution of $MgCl_2$ plus $MgSO_4$ and fresh water as
control, the record was 11.2 for the control and 34.2 for
the mixture. Again, in the case of KCl plus NaCl in
$m/10$ solution, the score was 31 for the control and 17
for the mixture. With double sea water (specific gravity
1.050) and ordinary sea water at 20° C., it was found
that the fish were attracted at the ordinary pressure and
temperature, reacting to the stronger solution an average
of 19.3 and to the control sea water 17.8 times. Further
experiments should be run to determine the influence of
antagonistic action of the salts in pairs. Whether or
not the results will coincide with the results of permea-
bility experiments will probably depend somewhat on the
factor of temperature (Loeb and Wasteneys, 1912).

### Influences other than Salts

The foregoing experiments indicate clearly that the
behavior of the fish under consideration is materially af-
fected by the salts with which they come in contact in
fresh water. However, the factors involved in the *migra-
tion* of fish are by no means thus explained. It is worthy
of note that the reactions of *Fundulus heteroclitus* to
toxic salts or even sewage are dependent on temperature
and stream pressure.

### Temperature

Numerous experiments were tried with varying tem-
perature and it was found that a temperature greater
than 23° C. repelled the fish and caused them to align
themselves along the current of fresh water at 20° C. in
preference to the slightly warmer sea water.

With a reduced temperature, even one degree less than
the control (19° C.), the fish were markedly attracted.
In fact it was possible to lure them into double sea water,
KCl or fresh water if these were presented at the proper
temperature. Further experiments and observations are
necessary for these and other species in order to deter-
mine the relation between gonad development, bodily
condition and the responses to temperature change.

As pointed out by Gurley (1902), the minnows migrate to warming water for the purpose of spawning, while the cod and the salmon migrate to cooling water for the same purpose. Chamberlain believes that the salmon come into water warmer than the sea water (1906).

Field records for *Fundulus heteroclitus* secured by the writer in connection with another investigation (1916) indicate the importance of temperature. The fish began coming inland in the spring when the water was about 15° C. and continued to run in and out until the inland pools had reached a temperature in August of about 24° C. Then for a period of over two weeks, they ceased running. About September 1, when the temperature had again lowered, they appeared again and continued to run until the temperature ran down to 10° C.

### Stream Pressure

When sea water was introduced through the ⅜-in. glass tube with a force sending it horizontally to a distance of 6 inches, while fresh water was introduced through the experimental tube into the adjoining trough with a force sending it 12 inches from the end of the tube, there was no difficulty in luring the fish away into the fresh water and keeping them directed towards it.

Many experiments were made, toxic substances such as KCl and double sea water also being introduced, but the increased pressure always proved the powerful factor. Chamberlain (1906), Prince (1920) and others have previously shown that in the case of the salmon, migration into fresh water is delayed until the floods come down into the bays and small streams. The arrival of a volume of rushing water furnishes the needed stimulus and the fish proceed forthwith to obey their instinct to swim against the current.

That fish can determine the presence of toxic substances in sea water or in fresh water is unquestionably demonstrable. But we have much evidence that those fish lying offshore and habitually migrating up a certain

stream, will journey into polluted water, spawn in places where the eggs can not develop and in many cases, die in such water themselves.

Salmon are reputed to return to the lake-fed streams where they were spawned and there is considerable evidence that they are guided by temperature difference, probably also by the current pressure, number of waterfalls, oxygen content and even by food. There is no question (Meek, 1916), however, that salmon ascend streams where no salmon could hitherto have spawned.

The destruction of protecting forests, spoliation of natural waterways and the utilization of streams by manufacturers wishing to dispose of wastes are the factors which not only cause the death of fish embryos and adults, but prevent the natural control of insect pests by their destruction in the larval state.

## SUMMARY

1. *Fundulus heteroclitus* is able to discriminate toxic from non-toxic salts at a temperature and stream flow the same as the control.

2. Variations in temperature or in stream flow profoundly influence the reactions and are more powerful factors in the behavior of the fish than presence or absence of salinity.

3. In the apparatus used, errors due to the notable reactions of fish to currents of water have been reduced by presenting the control and experimental flows parallel to each other.

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# SHORTER ARTICLES AND DISCUSSION

## NOTE ON ASSORTATIVE MATING IN MAN WITH RESPECT TO HEAD SIZE AND HEAD FORM

Assortative mating in man has been much discussed[1] but has been little investigated by scientific methods.

For characters such as age of husband and age of wife where there is an obvious preferential mating we may have coefficients of assortative mating as large as $r = +.75$. For stature, span and forearm Pearson has determined coefficients of about $+.20$ for span and span, $+.20$ for forearm and forearm, and $+.28$ for stature and stature in husbands and wives in his English series. The cross correlations for these various characters are in general smaller. For bodily characters other than stature the data are very few and are in general unsatisfactory.

With characteristic caution Pearson long ago suggested[2] that coefficients of assortative mating might be due to the husbands and wives being drawn from the same local races. The importance of this factor seems to be very small in his own materials.

This question must continually recur whenever assortative mating for physical characters is discussed. It seems very desirable, therefore, to obtain some measure of the correlation between husband and wife with respect to cephalic index, a character which has been considered of great importance by anthropologists in differentiating the races of Europe. For head size and head form we have had, as far as we are aware, until recently only the data for forty-eight families of Eastern European (Russian) Jews living in New York City, for which Boas[3] found assortative matings for cephalic index measured by $r = .15 \pm .10$.

Recently Frets in a series of papers[4] has given data for head

[1] The literature of the field has been reviewed up to 1912 by one of us: Harris, J. Arthur, ''Assortative Mating of Man,'' *Popular Science Monthly*, 80: 476–492. 1912.

[2] Pearson, K., ''Data for the Problem of Evolution in Man. III. On the Magnitude of Certain Coefficients of Correlation of Man,'' etc., *Proc. Roy. Soc.*, Vol. 66: 23–32. 1899.

[3] Boas, F., ''Heredity ir Head Form,'' *Amer. Anthrop.*, N. S., 5: 532. 1903.

[4] Frets, G. P., ''Heredity of Head Form in Man,'' *Genetica*, 3: 193–400. 1921. This paper contains the original measurements. These have been to some extent checked against his other papers.

length, head breadth and cephalic index in a series of Dutch families. He has himself calculated a coefficient of correlation of .039 ± .034 for the cephalic index of husband and wife in 389 families.[5] We have felt it desirable to determine the correlation for length and width of head, as well as that for index.

Because of a suggestion by Pearson (*loc. cit.*) that the correlation apparently indicating assortative mating may be really due in some cases to an association of fertility with homogamy, we have thought it desirable to calculate all the coefficients of correlation in two ways: (1) by using the actual number of parents, and (2) by weighting the parents with the number of offspring indicated in Frets' tables.[6]

The correlation coefficients are as follows:

Length of husband's head and length of wife's head:
Parents only, $r = +.0487 ± .0377$. $r/Er = 1.29$.
Parents weighted with their children,
$r = +.0616 ± .0376$. $r/Er = 1.63$.
Breadth of husband's head and wife's head:
Parents only, $r = +.1197 ± .0372$. $r/Er = 3.22$.
Parents weighted with their children,
$r = +.1184 ± .0372$. $r/Er = 3.18$.
Index of husband's head and index of wife's head:
Parents only, $r = +.0231 ± .0377$. $r/Er = 0.61$.
Parents weighted with their children,
$r = -.0546 ± .0376$. $r/Er = 1.44$.

The constants are with one exception positive in sign. That for the breadth of husband and breadth of wife may perhaps be considered statistically significant in comparison with its probable error. The others, particularly that for the cephalic index, can not be so considered.

The coefficients may, therefore, indicate a slight assortative mating for the dimensions of the head. The coefficients, in common with those for physical characters other than stature, are relatively low. That the correlation for the cephalic index is so low is a point of particular interest. If cephalic index be a character of great importance in distinguishing races, and if correlations which have been demonstrated between the physi-

[5] Frets, G. P., "Erfelijkheid, correlatie en regressie," *Genetica*, 3: 1–27. 1921.

[6] We are able to abstract from Frets' tables 319 pairs of parents in which there were no indications of typographical errors when different tables were checked against each other. These had a total of 1328 recorded children. In calculating the probable errors of the coefficients we have used the unweighted number of parents as $N$.

cal characteristics of husband and wife be due primarily to the
tendency to marry within the same racial group, one might ex-
pect a large correlation for cephalic index. Instead we find
the lowest correlation of the three determined.

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## A GYNANDROMORPH IN DROSOPHILA MELANOGASTER [1]

In 1916 Hyde and Powell described a mosaic female with
one eye eosin and the other blood. They interpreted this case
in the light of Morgan's suggestion of 1914 that "Gynandro-
morphs and mosaics may arise through a mitotic dislocation of
the sex chromosomes." In other words they believed one X
chromosome carrying the gene for eosin went into the cells of
one eye and the other X chromosome carrying the gene for
blood went into the other eye. In 1919 Morgan and Bridges
described a large number of gynandromorphs. The hypothesis
of chromosomal elimination explains most of them, but a num-
ber of special cases are explained in other ways. One of their
special cases was a male with one eye eosin and the other eosin
vermilion. They explained this case by assuming that the egg
had two nuclei, one of which after maturation had an eosin
vermilion X chromosome and the other an eosin X chromosome.
Further, they assumed each nucleus to have been fertilized by
a Y sperm. These hypotheses would explain the facts that the
individual was male throughout and that one eye was eosin
vermilion and the other eosin.

In our experiments a somewhat similar mosaic appeared.
The individual was made throughout, with one eye garnet and
one white. The parentage was as follows: a garnet male was
mated to a yellow white female. An $F_1$ wild-type daughter
was mated to an $F_1$ yellow white male. From this pair of
parents the mosaic arose. It was fertile and was bred to a
garnet female. In $F_1$ all males and females were garnet. The
$F_1$ garnet males and females were inbred. In $F_2$ the females
were garnet but the males were garnet and white in approxi-
mately equal numbers (1,089 garnet to 1,026 white). This
demonstrates very clearly that the mosaic was genetically a

[1] Zoological Laboratory Contribution No. 191. Indiana University.

garnet white. Professor Morgan writes us that he would also interpret this case on the binucleated egg hypothesis. We see clearly how the hypothesis may be applied and that the binucleated eggs described by Doncaster may give indirect evidence in its favor. Perhaps it is the best interpretation. We wish to point out, however, that there are other possibilities although they may have no direct or indirect morphological or experimental evidence in their favor.

Let us assume the individual started as a normal male, the single X chromosome carrying the genes for garnet and white. Since the mosaic did not carry the gene for yellow, the garnet white genes must have been brought together by a double crossing over in the mother. The only assumption we need to make is that during somatogenesis, the white end of one of the daughter X chromosomes became in some way inactive or lost. This would leave in one cell a whole X chromosome carrying white and garnet; in the other an imperfect X chromosome carrying garnet only. We know by test that white and garnet in the same chromosome give an eye practically indistinguishable from white. If one eye arose from the descendants of one of these two cells and the other eye from the second cell, we could account for the difference in color. The only assumption we need to make then is the loss or inactivation of the white gene in one of the early cleavage cells. On the binucleated egg hypothesis we must assume, first, the presence of two nuclei within the egg; secondly, that each nucleus is fertilized by a Y sperm; and thirdly, that the sex cells of the male arose from the descendants of only one of these nuclei, as all sperm were alike, carrying garnet and white.

A second possibility is that of somatic mutation. If the white gene in one of the cells should mutate to red, we would have a cell whose X chromosome carried the gene for garnet. If the descendants of this cell gave rise to one eye and the descendants of the other cells to the second, we would have one eye garnet and one garnet white, which is white. It is true that white eye has never reverted to red in all the thousands which have been bred. This fact renders this suggestion improbable but not impossible.

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